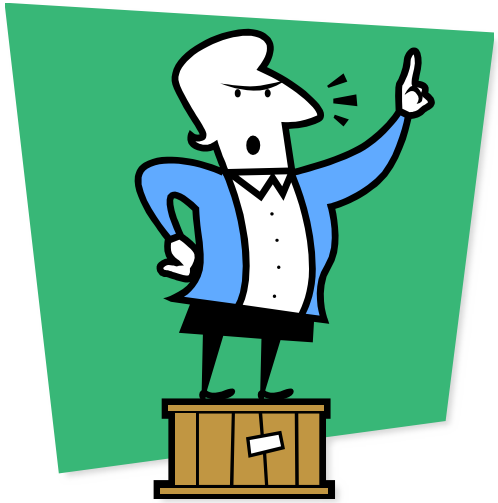
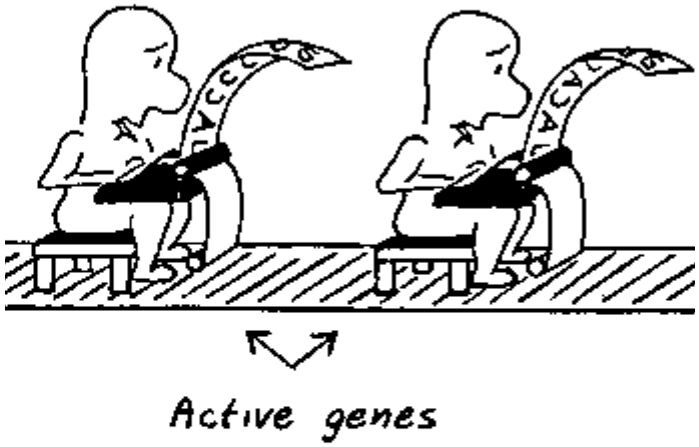
Thema der Vorlesung

Regulationsmechanismen in Bacteria und Archaea

Sensen von Umweltsignalen

- DNA Bindeproteine
- Operonkonzept
- Lac-Operon
- Katabolitregulation
- Attenuation (Schleifenbildung)





The genes of procaryotic cells are not allowed to sleep. Compared to eucaryotic cells, their activity is regulated by methods which are rather crude, but effective.

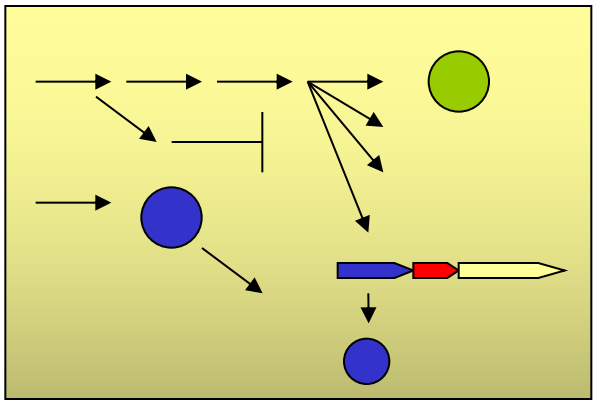
Signaltransduktion und metabolische Antwort auf Veränderungen (Limitationen)

N-Quelle

C-Quelle

O_2

Signaltransduktion



Unterschiedliche(r)

Metabolismus
Morphologie

Regulatorisches Netzwerk

Proteine, Modifikation von Enzymaktivitäten
Transkription
Translation

Einige Regulatorische Prinzipien und Ebenen:

Proteine: Modifikation von Enzymaktivitäten

- **posttranslationale Modifikationen**
  - reversibel (Phosphorylierung,Adenylierung…)
  - permanent (Abbau; Oxidation von FeS-Zentren)
- **allosterische Veränderungen**

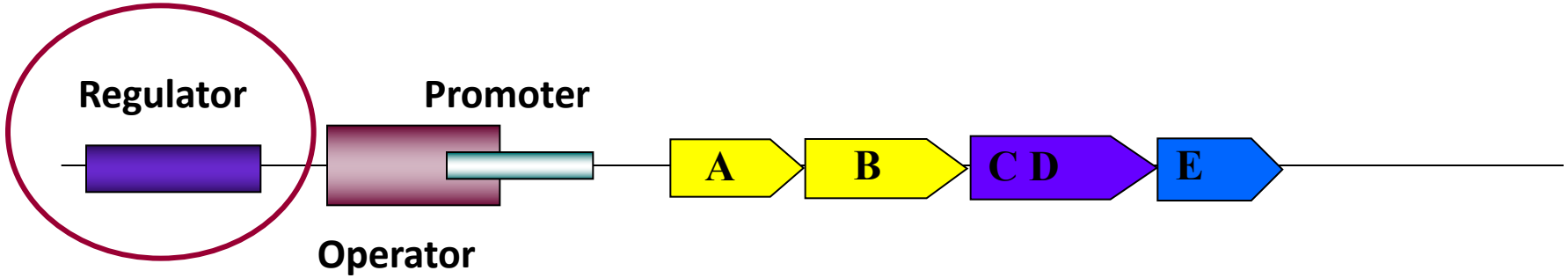
Transkription

-positiv: Aktivierung
-negative: Repression
-ncRNAs
-Riboswitches

Translation

-ncRNAs
-Riboswitches
-Attenutation (Trp-Operon)

Operonkonzept



- Prokaryoten
- Serie von hintereinandergeschalteten Genen
- Alle Gene in einer einzigen mRNA transkribiert:

polycistronische mRNA

Standardtechniken zur Messung von Genexpression und Regulation:

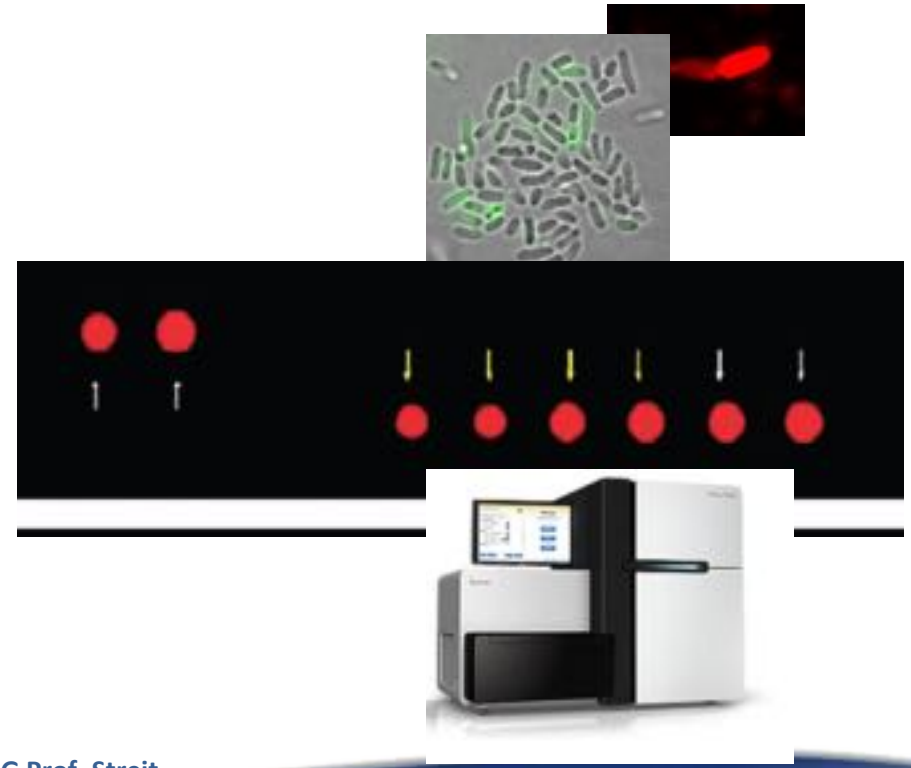
Einzelgene:

Reporter-Genfusionen
Zielgen (Promotor) mit Reportergen verknüpft

Messung dann per Küvette,
Fluoreszenz in Mikrotiterplatte etc..

Alle Gene/ORFS einen Organismus

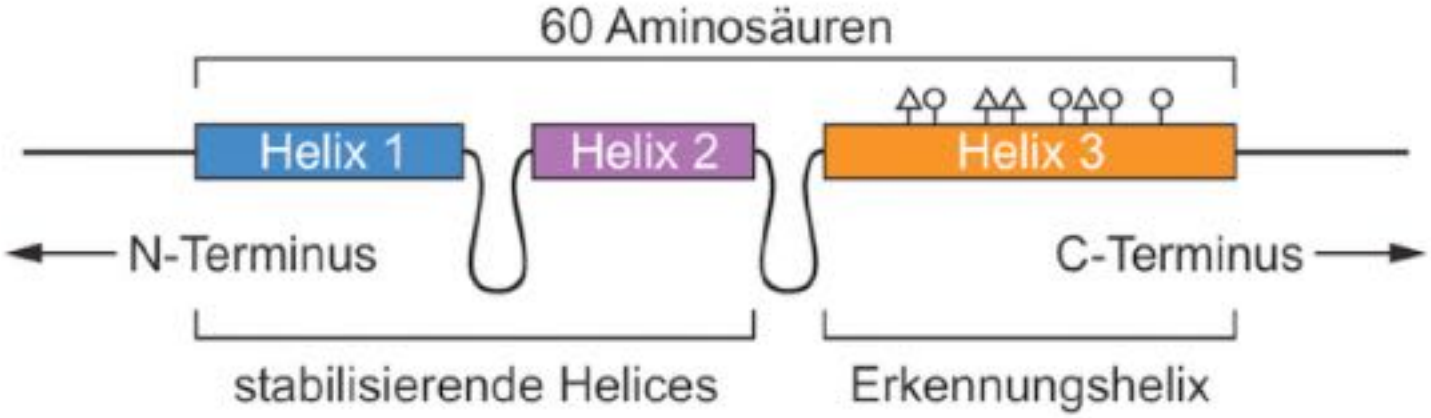
Microarrays

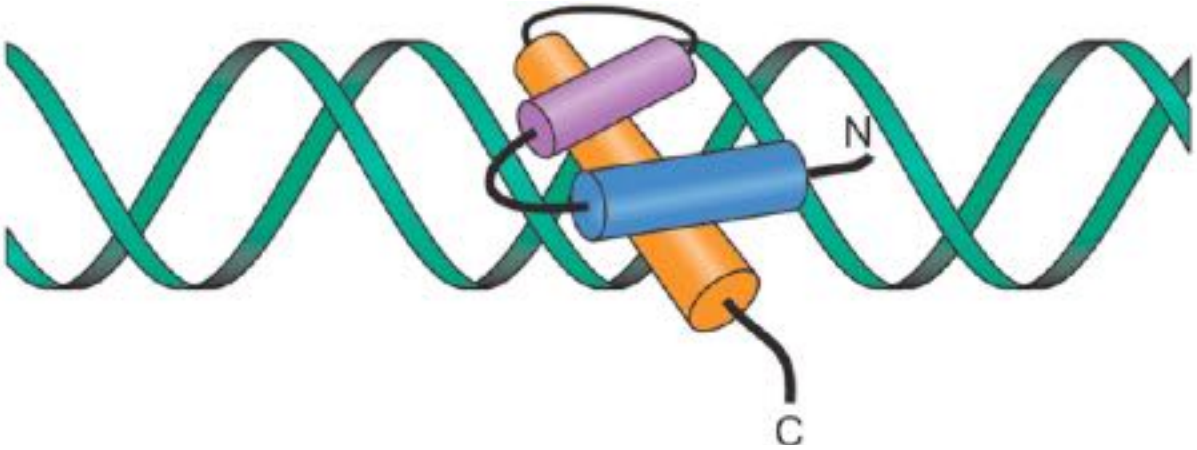


RNA-Seq-Technologie

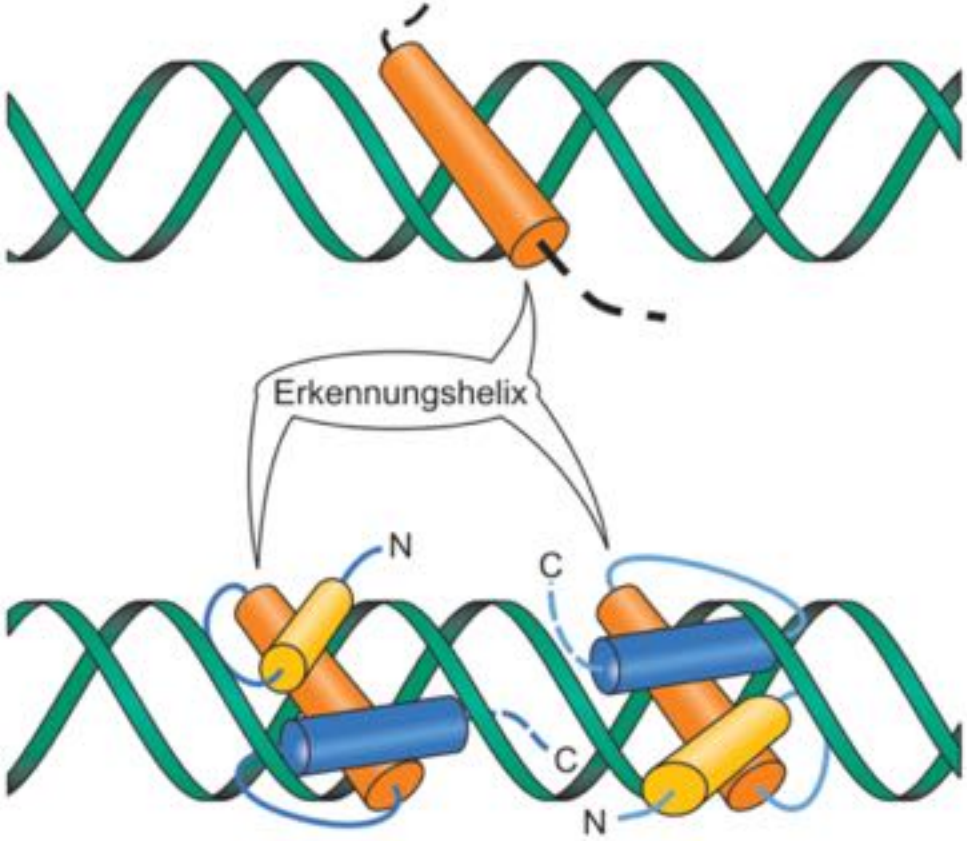
DNA-bindende Proteine

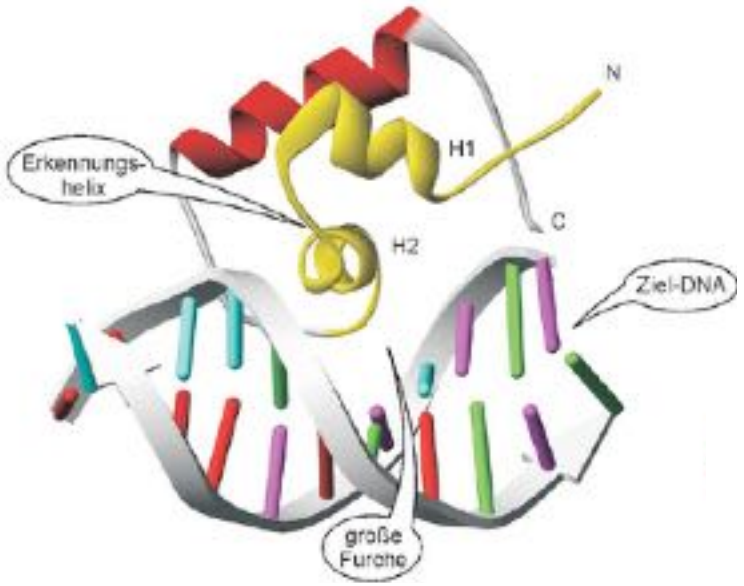
Helix-turn-helix Motif



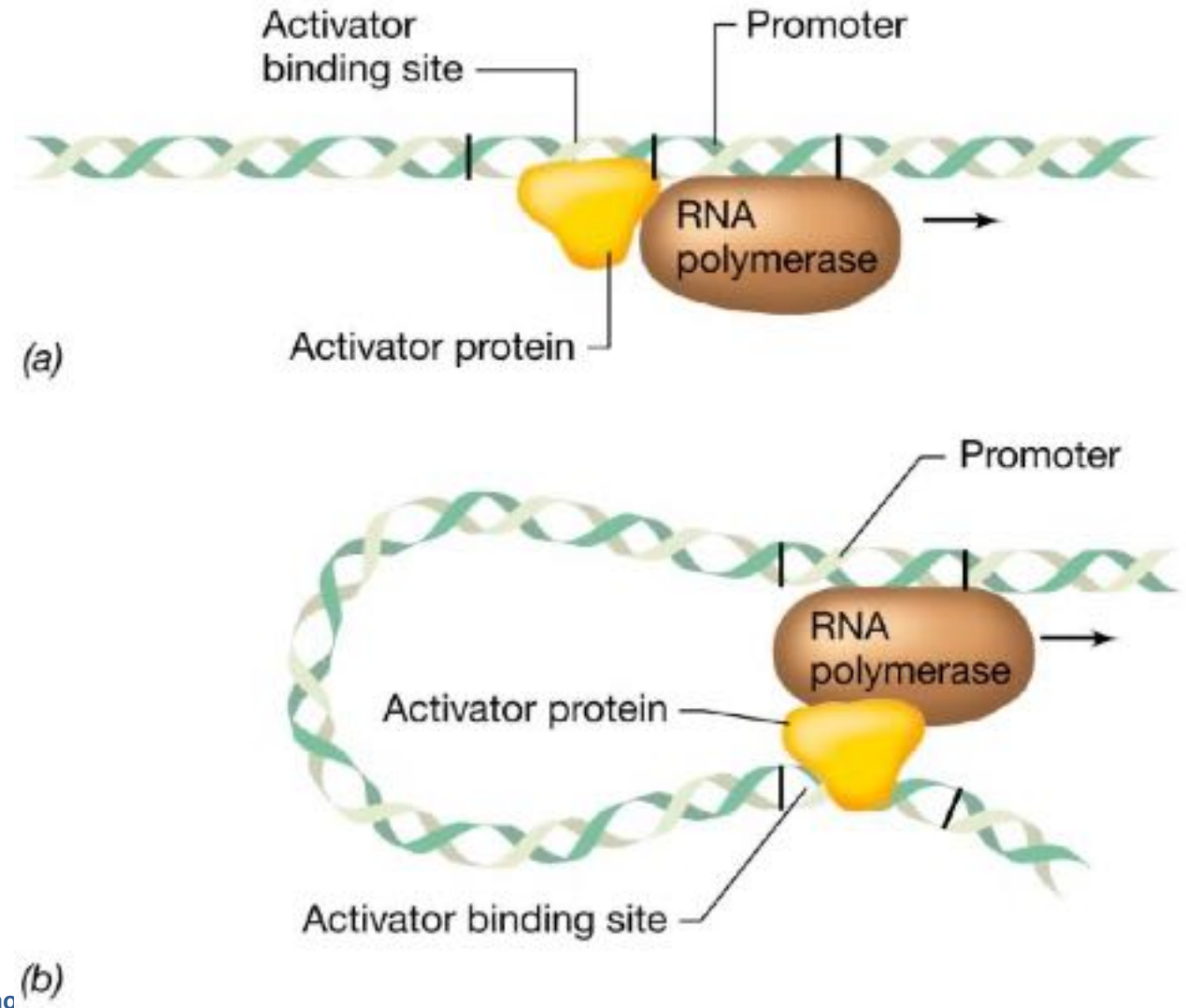


DNA-bindende Proteine

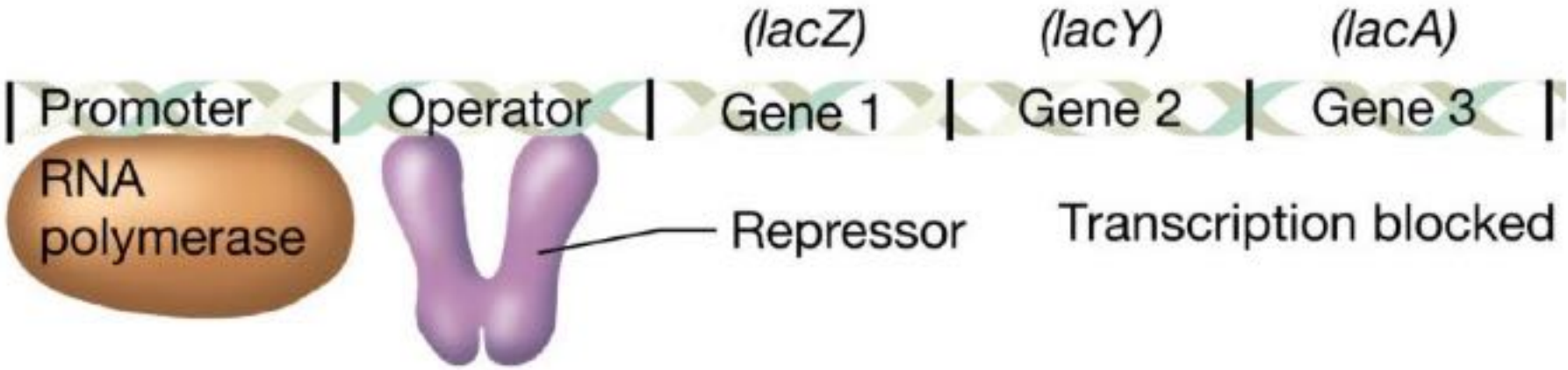




Aktivator


Activator
binding site
Promoter
RNA
polymerase
Activator protein
(a)
Promoter
RNA
polymerase
Activator protein
Activator binding site
(b)

Repressor


(lacZ)
(lacY)
(lacA)
Promoter
Operator
Gene 1
Gene 2
Gene 3
RNA polymerase
Repressor
Transcription blocked

Jaques Monod
1910-1976

ENZYMATIC ADAPTATION AND ALLOSTERIC TRANSITIONS

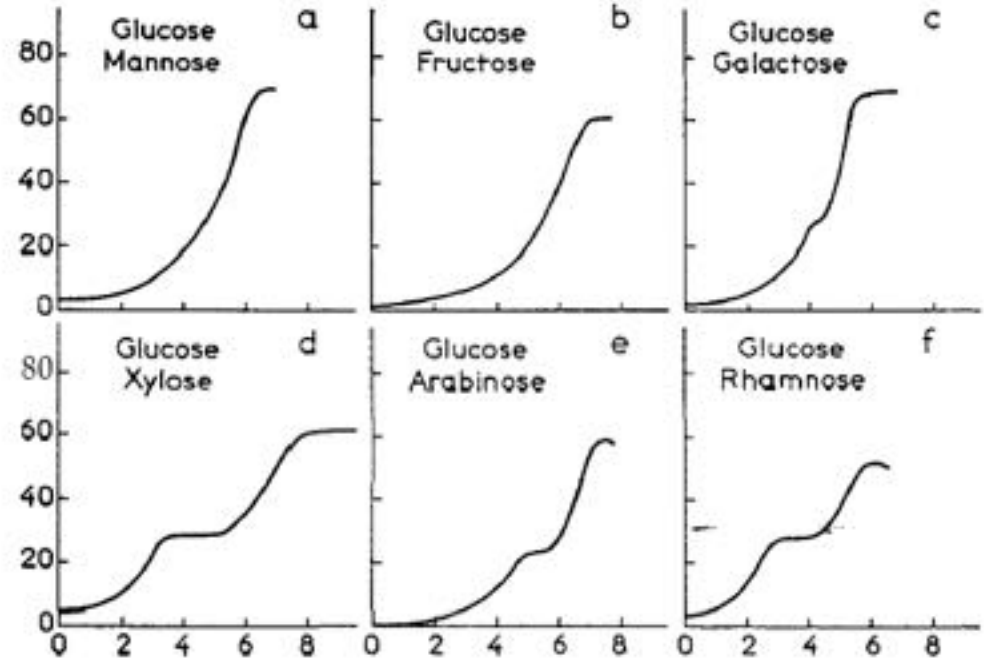
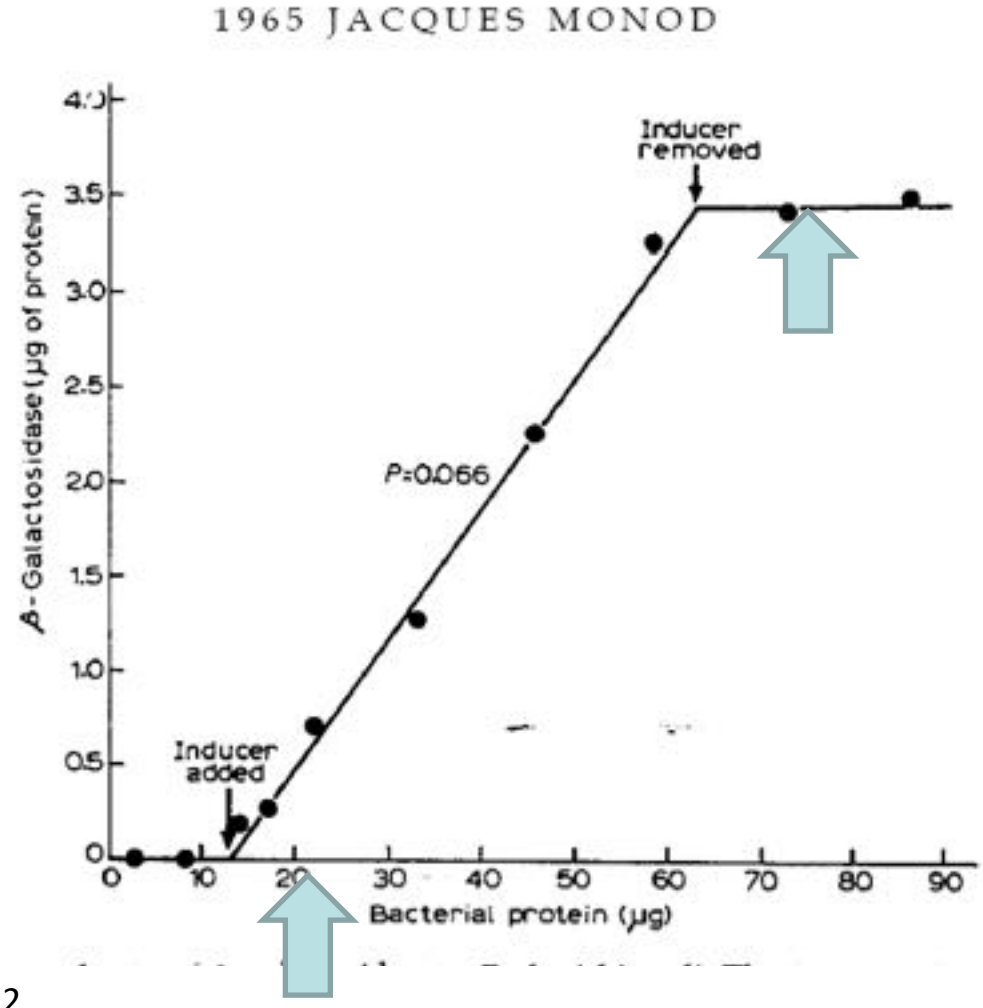
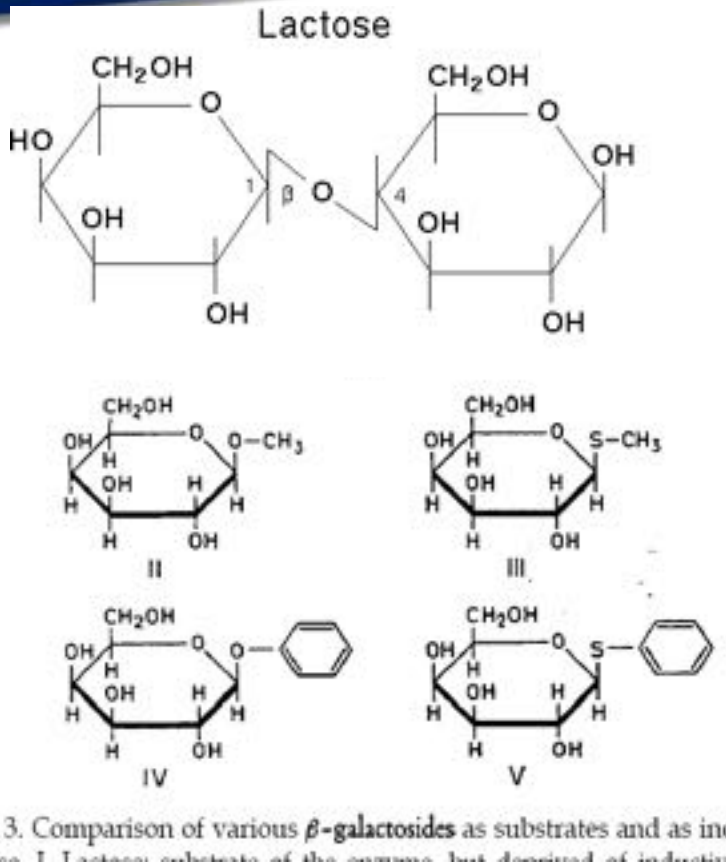


Fig.1. Growth of *Escherichia coli* in the presence of different carbohydrate pairs servi the only source of carbon in a synthetic medium[30].

Diauxie: Wachstum von *E. coli* in einem Gemisch aus Glucose und anderen C-Quellen; immer erst die einfacher zu verstoffwechselnde C-Quelle abbauen – Regulationsprinzip!

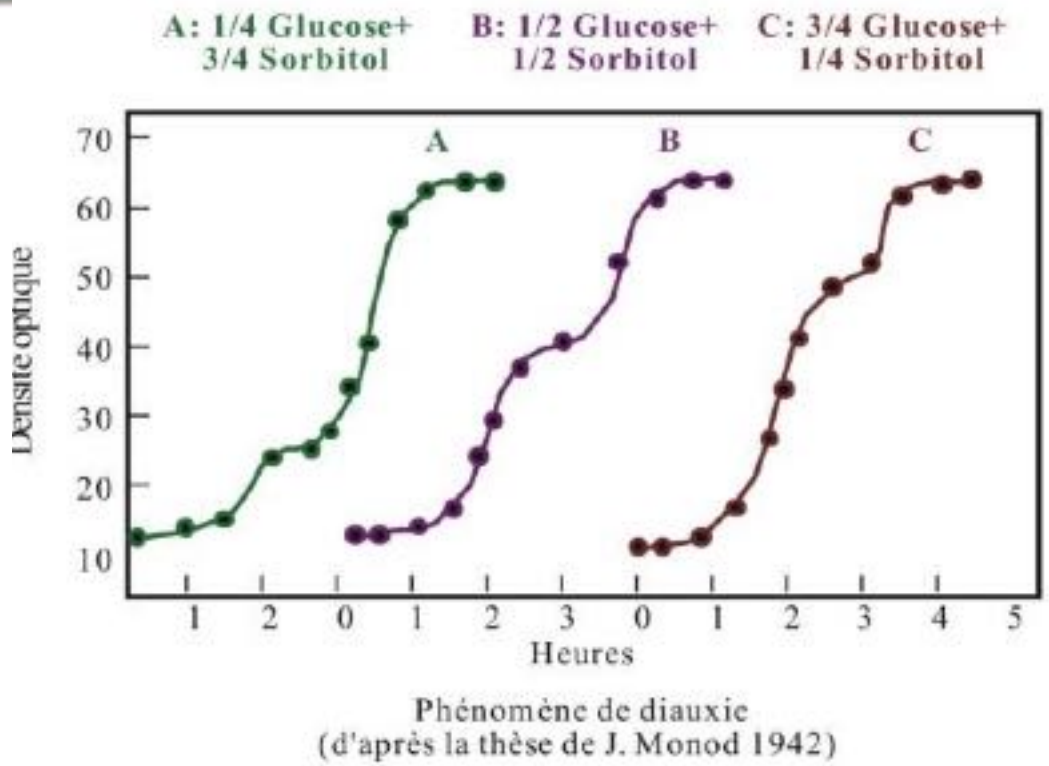




3. Comparison of various β-galactosides as substrates and as inducers

Induktionsversuche zur ß-Gal-Synthese und Induktoren

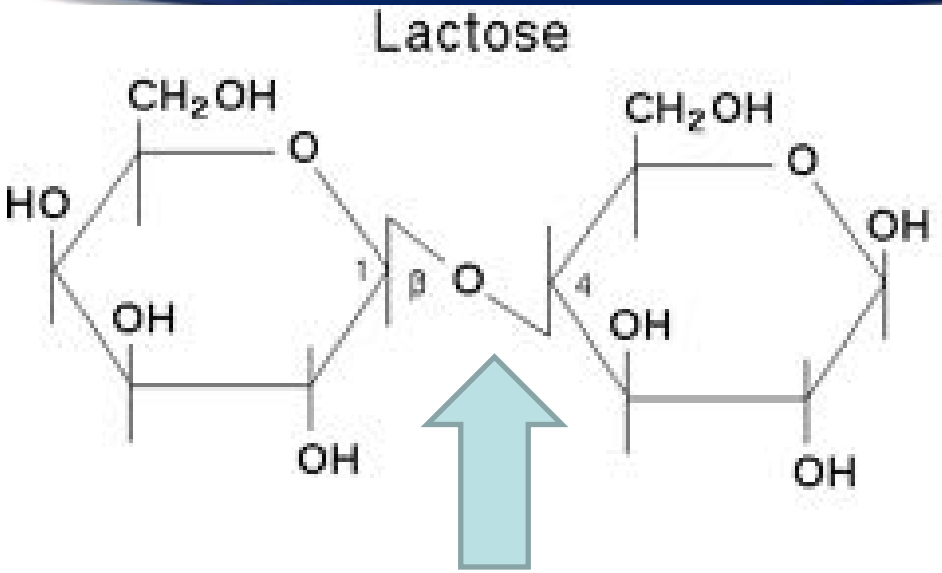
Diauxie-Katabolitrepression:

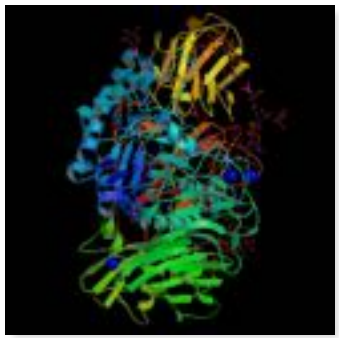


Phénomène de diauxie
(d'après la thèse de J. Monod 1942)

Diauxie: In Gegenwart mehrerer Kohlenhydratquellen werden zunächst die energie-reichsten Substrate verwertet; J. Monod (1942)

Katabolitrepression: Anwesenheit einer bevorzugten Kohlenhydratquelle verhindert die Synthese von Enzymen für die Verwertung anderer Kohlenhydrate; B. Magasanik(1961).





ß-D-Galactose + ß-D-Glucose,

ß-1-4 verknüpft;

MW 342.3 g/mol.

$C_{12}H_{22}O_{11}$

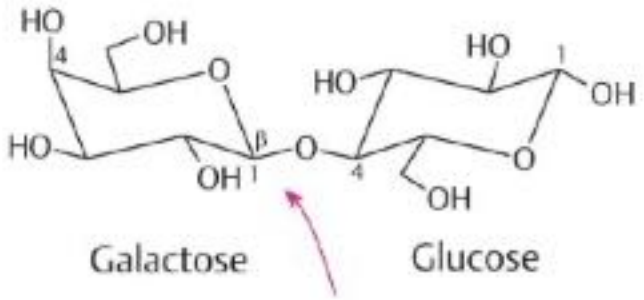
2-8 % in Milch enthalten

Induktor beim Lac-Operon:
Allolaktose Glc -ß-1,6-Gal; entsteht aus Transferasenebenaktivität der ß-Galactosidase

ß-Galactosidase: Trivialname Lactase, aktiv als Tetramer ca. 1000 aa; wird aus 2 Proteinen aufgebaut, kodiert LacZ und LacΩ

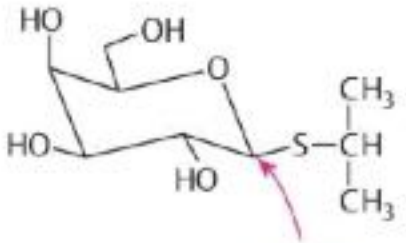
Lac-Operon

Lactose



β-galactosidische Bindung; wird durch das Enzym β-Galactosidase gespalten

Isopropyl-thiogalactosid



Diese Bindung kann nicht durch *E. coli*-Enzyme gespalten werden

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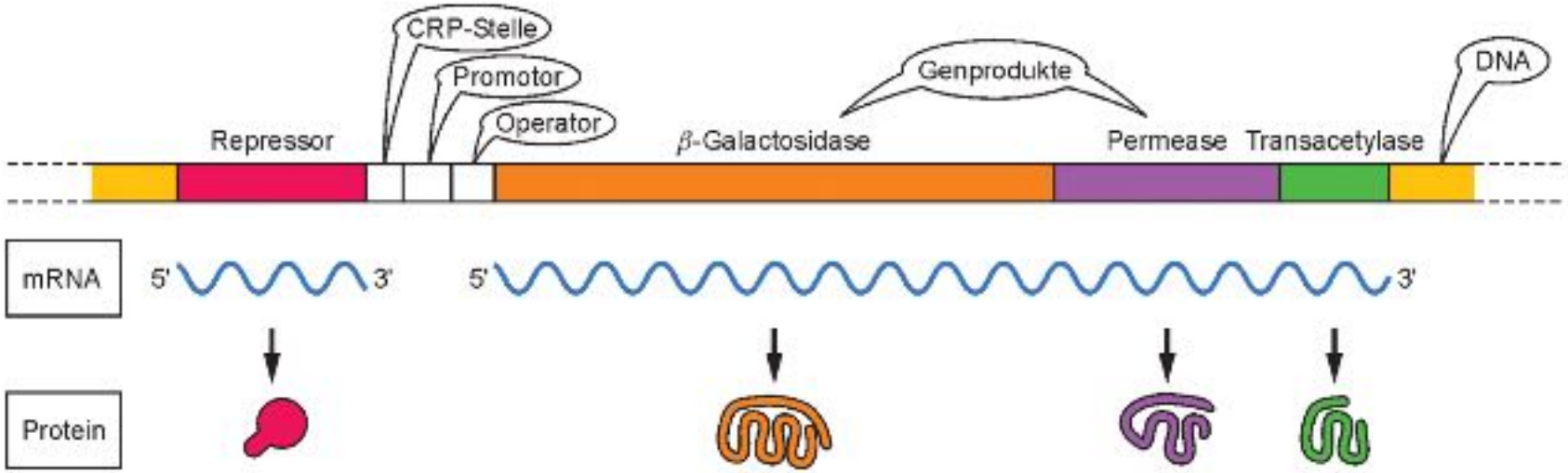
Lac-Operon

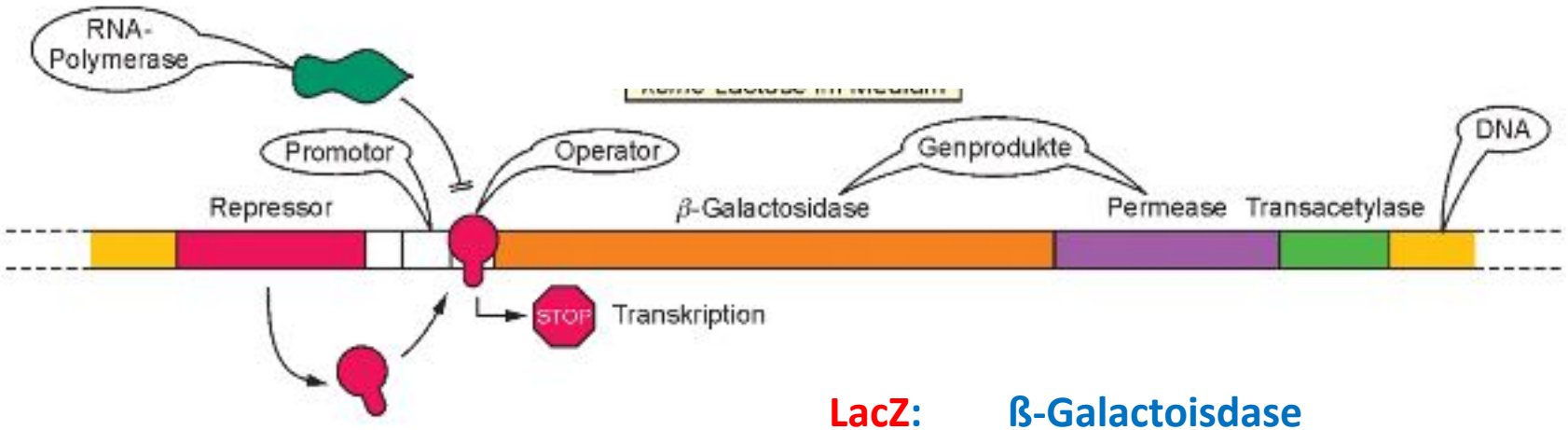
LacZ: ß-Galactoisdase
LacY: Permease
LacA: Acetylase

LacI: Repressor (sitzt upstream)



Lac-Operon

Transkriptionsinhibierung,
wenn keine Laktose im Medium

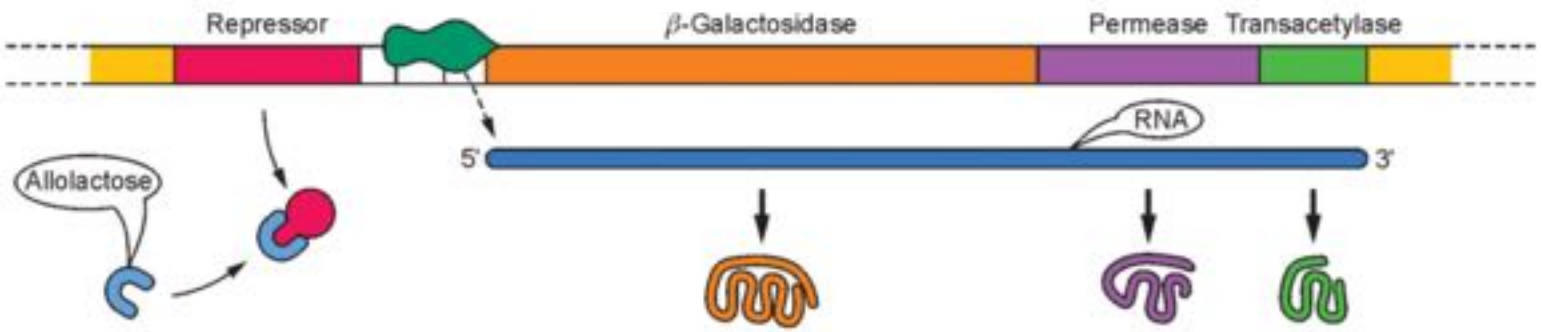


LacZ: ß-Galactoisdase
LacY: Permease
LacA: Acetylase

LacI: Repressor (sitzt upstream)

Lac-Operon

Transkriptionsaktivierung, wenn Laktose im Medium



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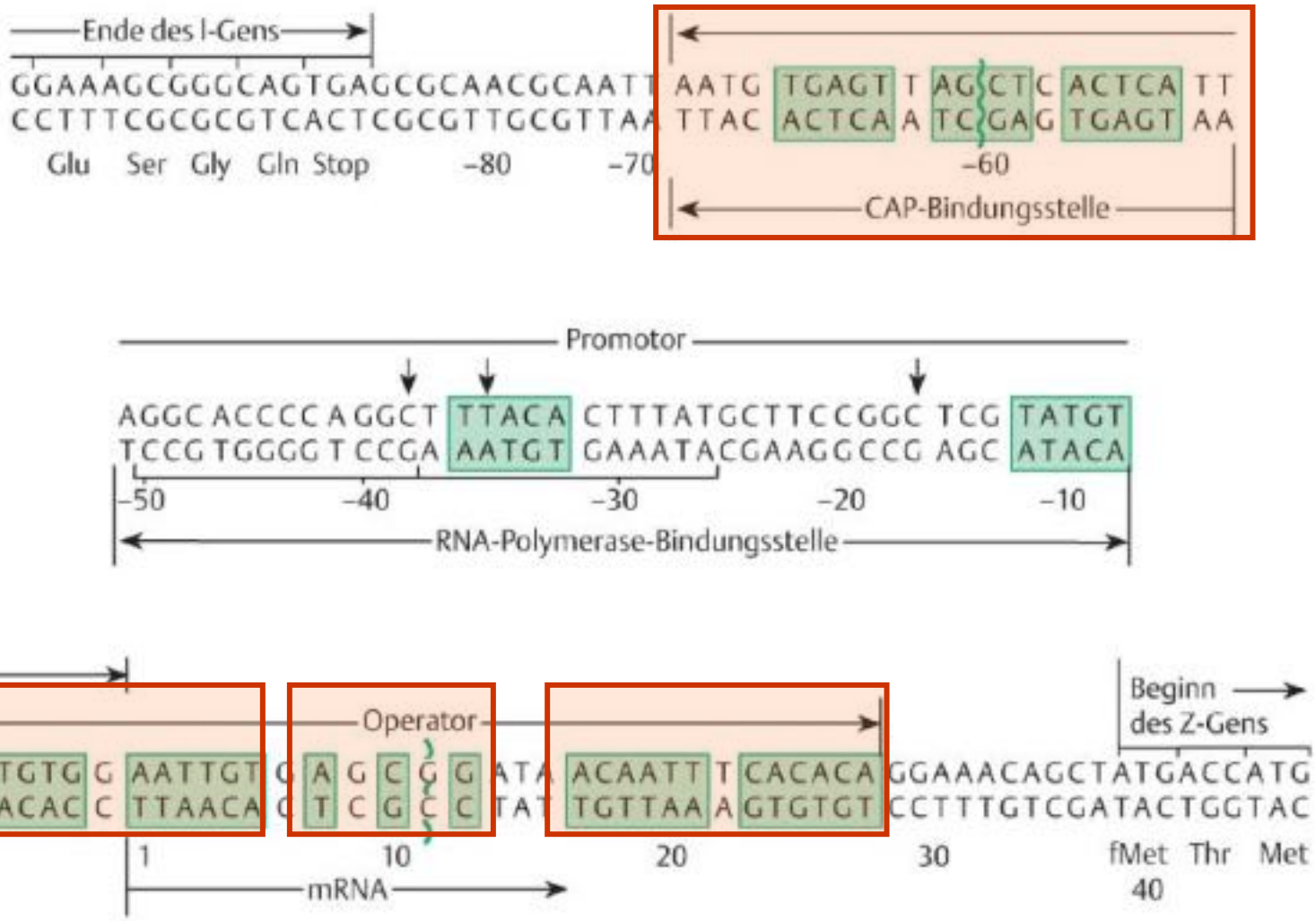
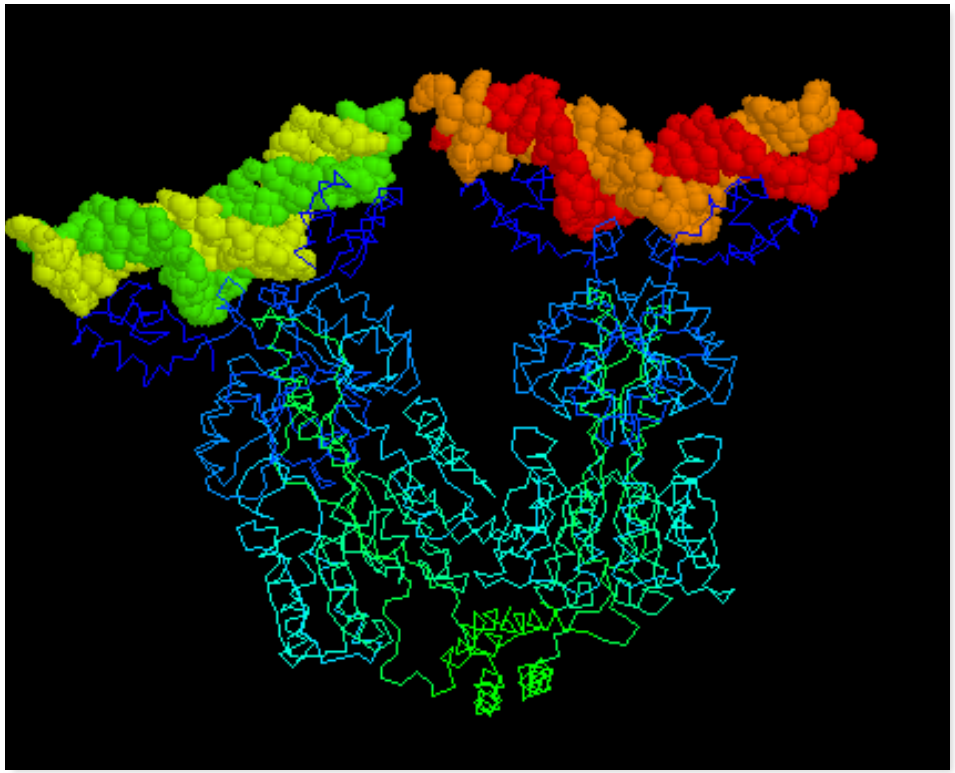
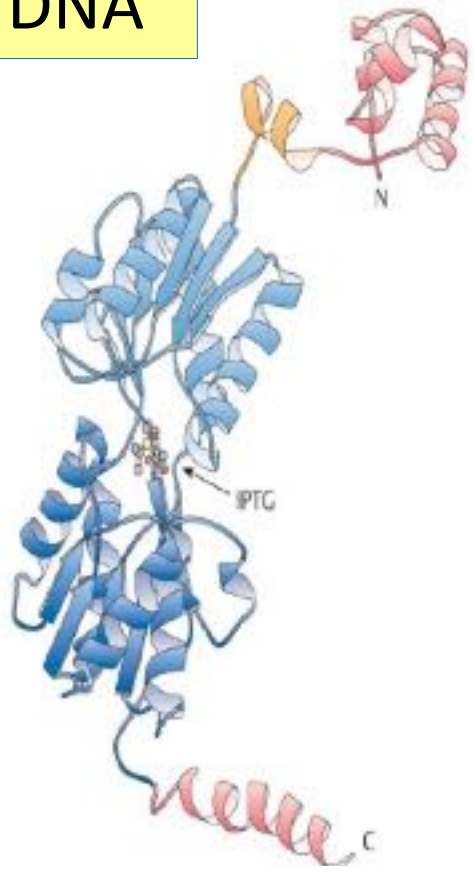
Lac-Operon


Ende des I-Gens
GGAAAGCGGGCAGTGAGCGCAACGCAATT
CCTTTCGCGCGTCACTCGCGTTGCGTTAA
Glu Ser Gly Gln Stop
–80
–70
AATG TGAGT T AG CT C ACTCA TT
TTAC ACTCA A TC GA G TGAGT AA
–60
CAP-Bindungsstelle
Promotor
AGGC ACCCC A GGCT TTACA CTTTATGCTTCCGGC TCG TATGT
TCCG TGGGG T CCGA AATGT GAAATACGAAGGCCG AGC ATACA
–50
–40
–30
–20
–10
RNA-Polymerase-Bindungsstelle
Operator
Beginn
des Z-Gens
TGTGTG G AATTGT G A G C G G ATA ACAATT T CACACA GGAAACAGCTATGACCATG
ACACAC C TTAACA C T C G C C TAT TGTTAA A GTGTGT CCTTTGTCGATACTGGTAC
1
10
20
30
fMet Thr Met
40
mRNA

Structure of the ***Lac Repressor*** bound to DNA

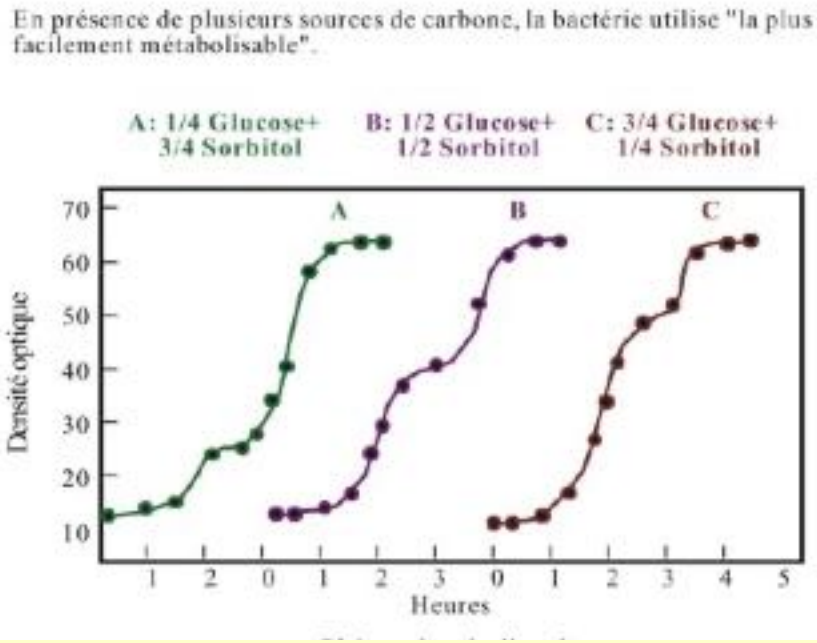




at 2.6 A resolution

Katabolitrepression: → Diauxie

Jaques Monod
1910-1976



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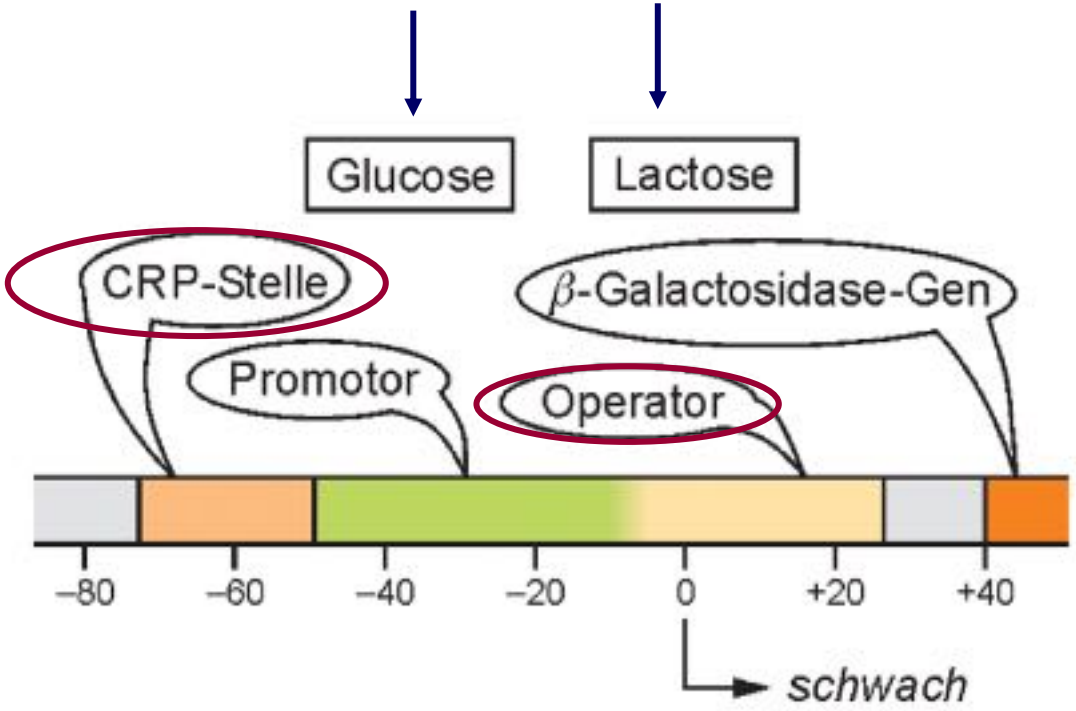
Katabolitrepression: Anwesendheit einer bevorzugten Kohlenhydratquelle verhindert die Synthese von Enzymen für die Verwertung anderer Kohlenhydrate; B. Magasanik(1961).

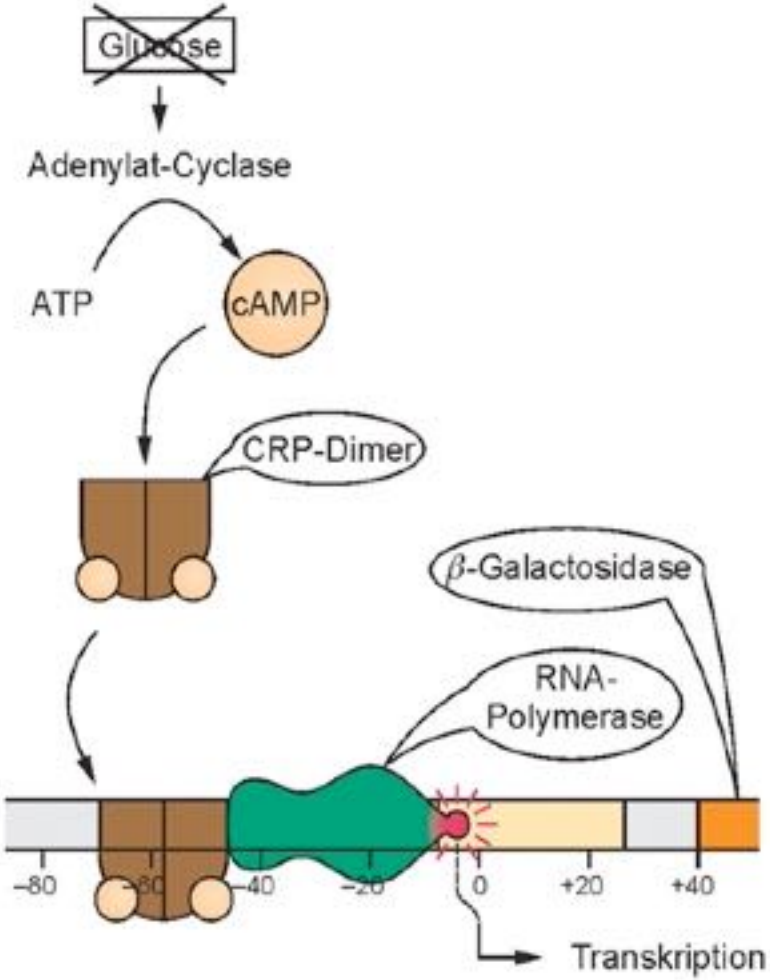
Lac-Promoter Katabolitrepression:

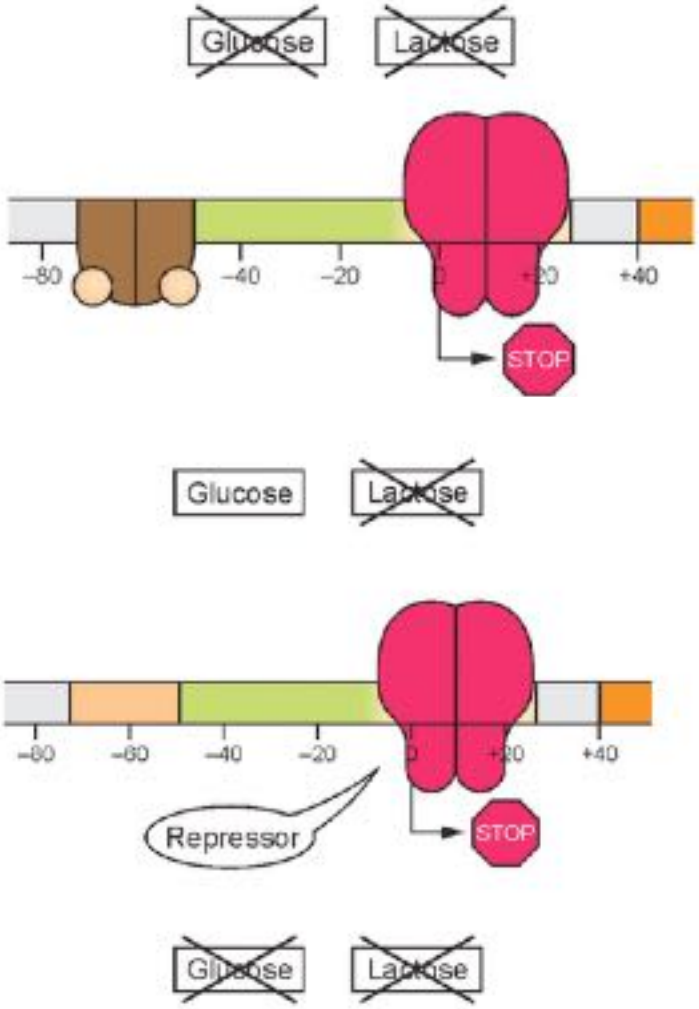
Signal: Glukose Abwesenheit

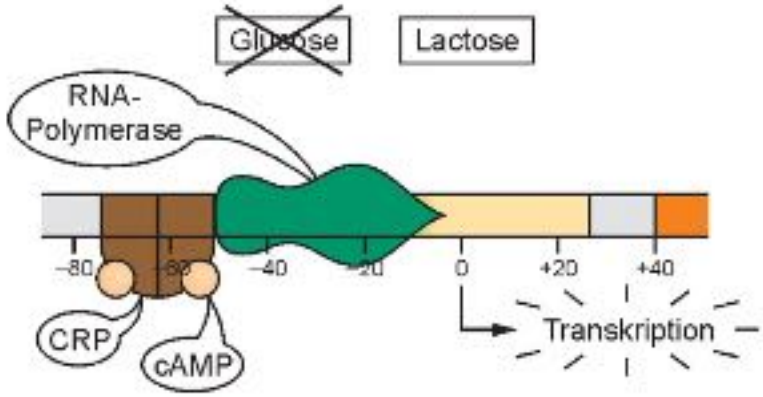
Signal: Laktose Anwesenheit



Lac-Promoter Katabolitrepression:





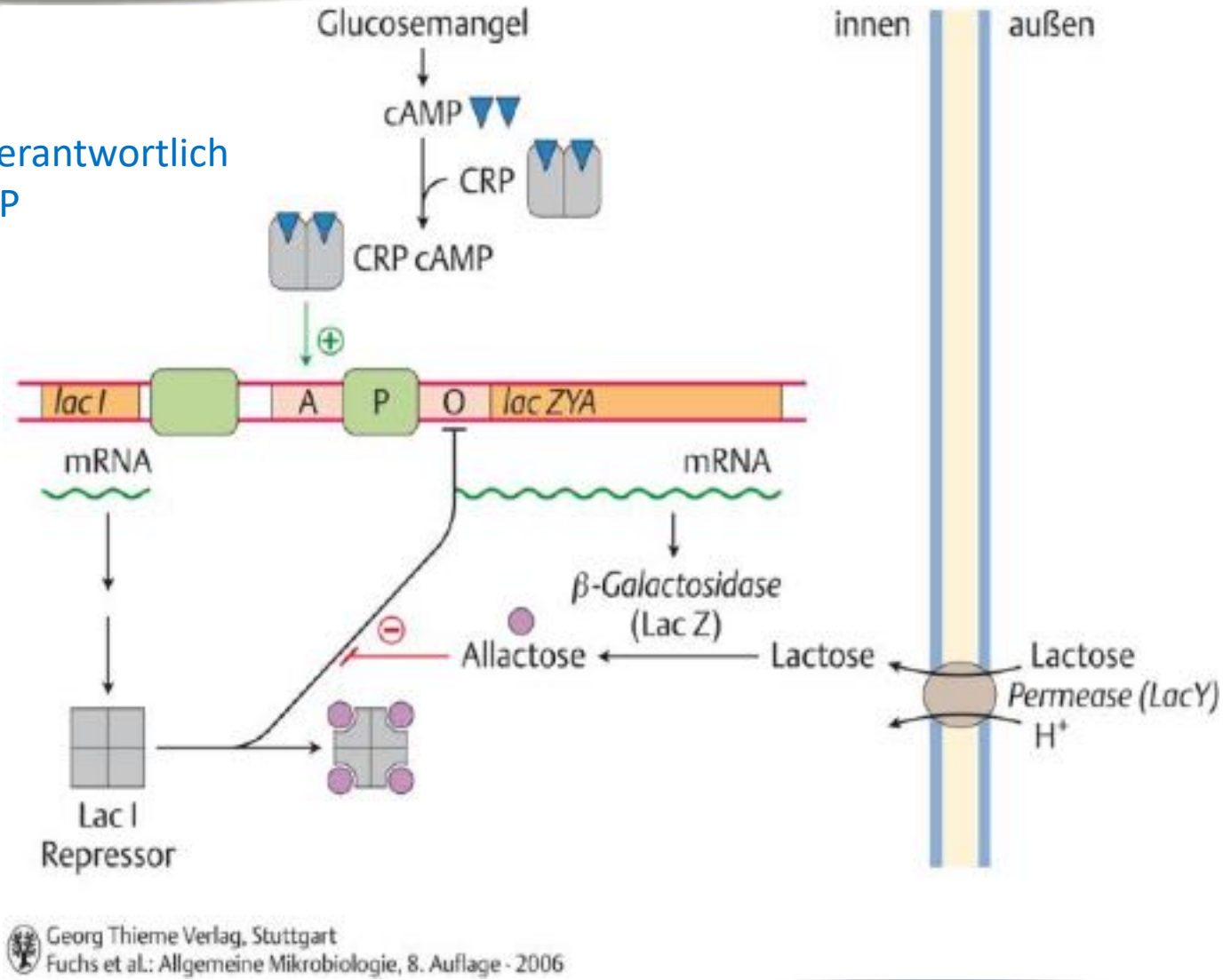


Lac-Promoter Katabolitrepression

Lac-Operon

eine Adenylatzyklase ist verantwortlich für die Synthese von cAMP

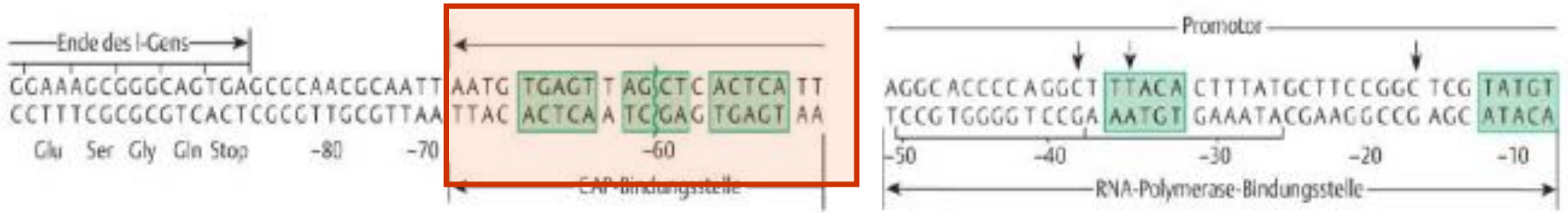
wenn keine Glukose vorliegt, dann hoher cAMP Spiegel, Interaktion mit CRP und volle Transkription der Lac-Gene

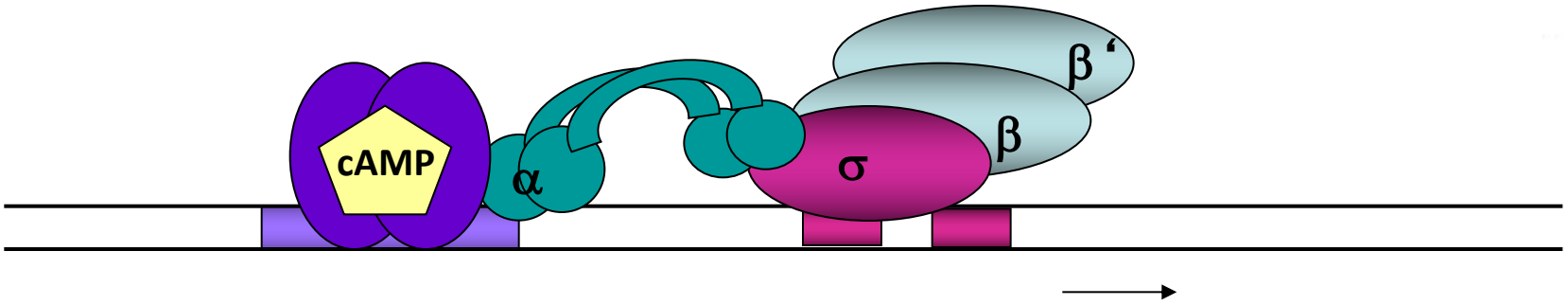


Georg Thieme Verlag, Stuttgart
Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage - 2006

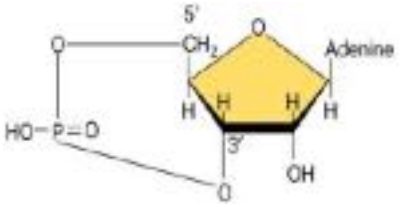
CAP-RNA Pol interaction

Ende des I-Gens
GGAAAGCGGGCAGTGAGCCCAACGCAATT AATG TGAGT T AG CTC ACTCA TT
CCTTTCGCGCGTCACTCGCGTTGCGTTAA TTAC ACTCA A TC GAG TGAGT AA
Glu Ser Gly Gln Stop -80 -70 -60
CAP-Bindungsstelle


Ende des I-Gens
GGAAAGCGGGCAGTGAGCCCAACGCAATT AATG TGAGT T AG CTC ACTCA TT
CCTTTCGCGCGTCACTCGCGTTGCGTTAA TTAC ACTCA A TC GAG TGAGT AA
Glu Ser Gly Gln Stop -80 -70 -60
CAP-Bindungsstelle
Promotor
AGGC ACCCC AGGCT TTACA CTTTATGCTTCCGGC TCG TATGT
TCCG TGGGG TCCGA AATGT GAAATACGAAGGCCG AGC ATACA
-50 -40 -30 -20 -10
RNA-Polymerase-Bindungsstelle



CAP site **-35** **-10** **+ 1**



cAMP

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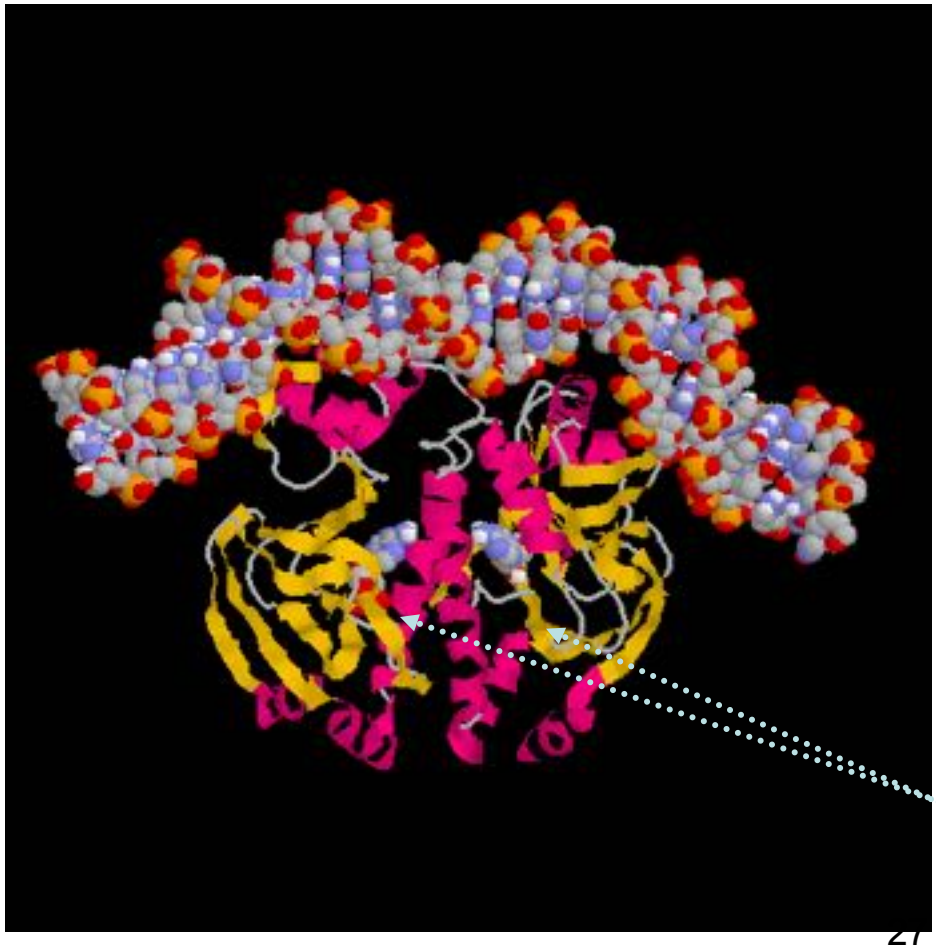
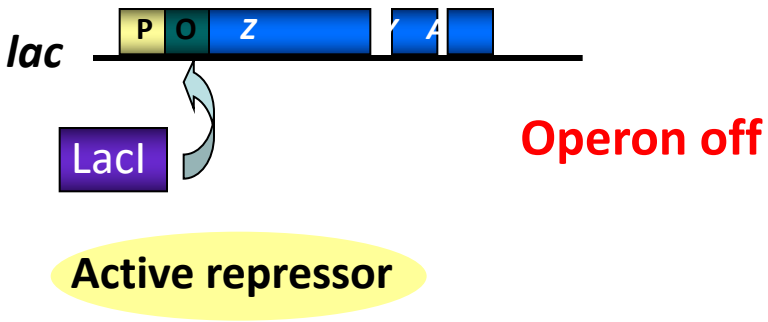
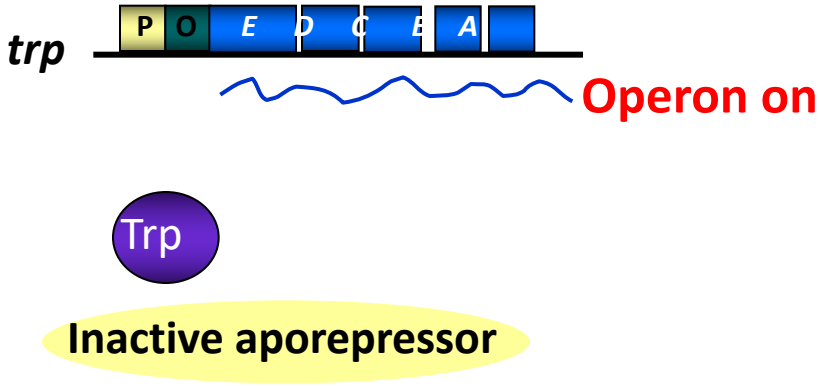
Structure of the CRP-cAMP complex bound to DNA

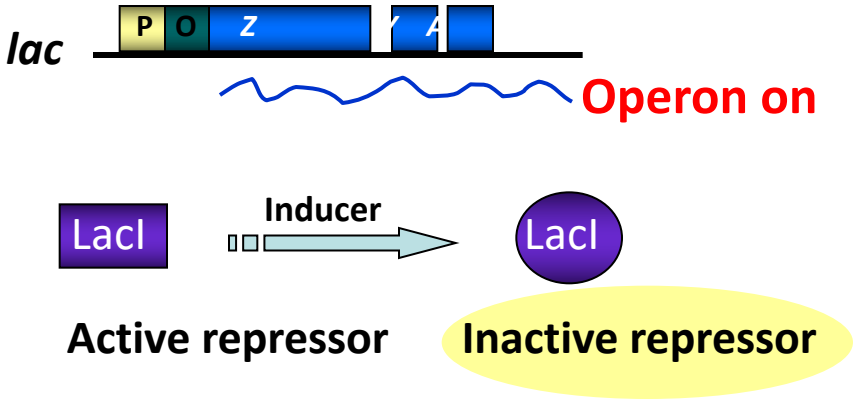


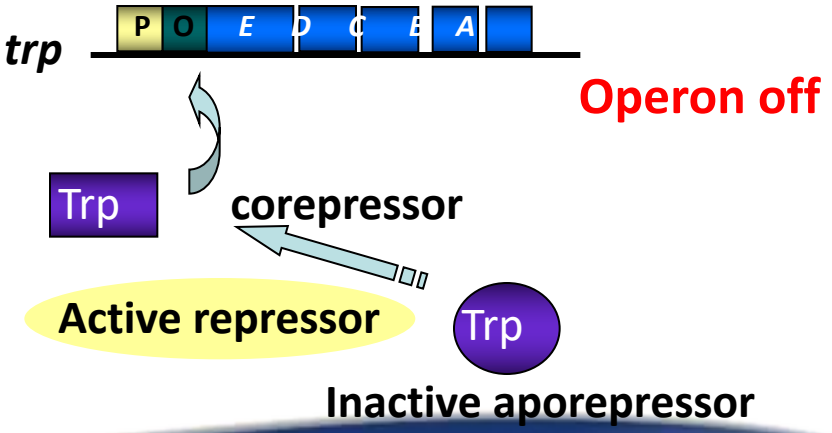
Negative control (*lac* operon)



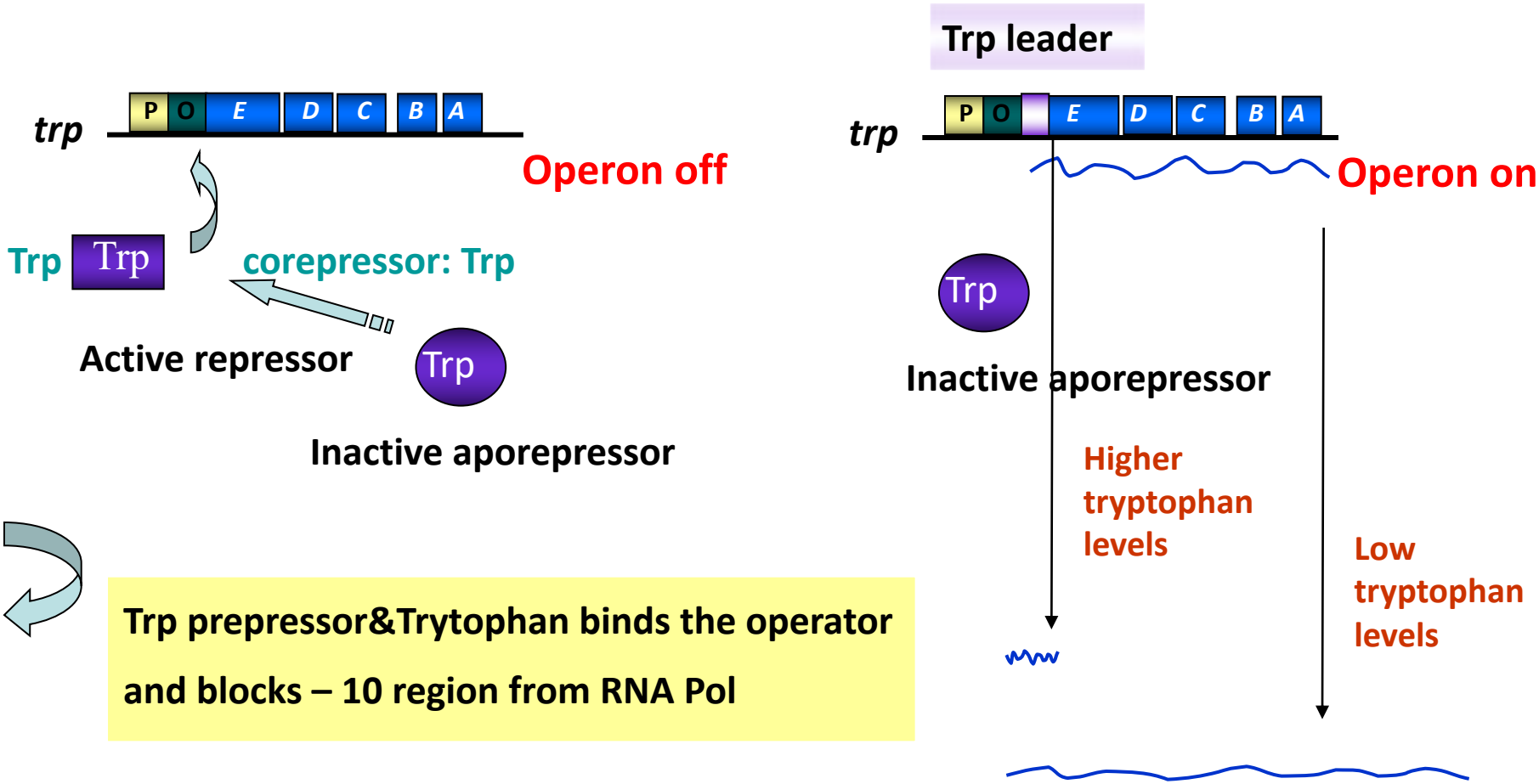
Negative control (*trp* operon)





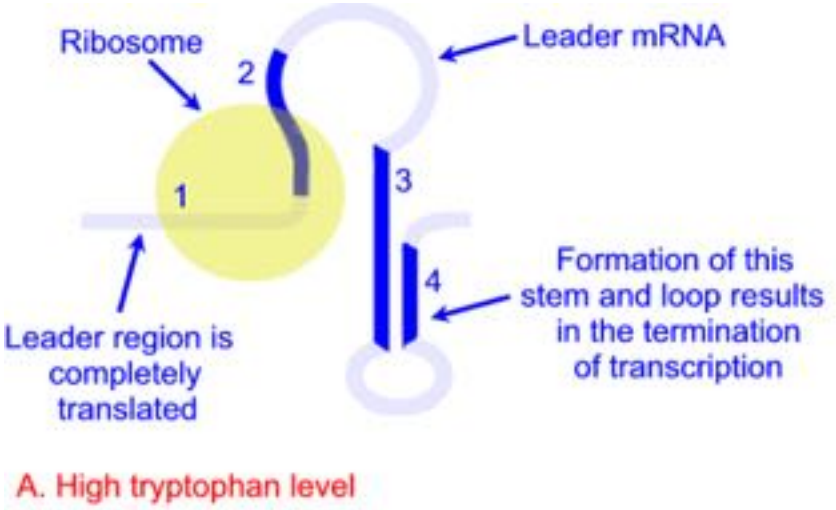


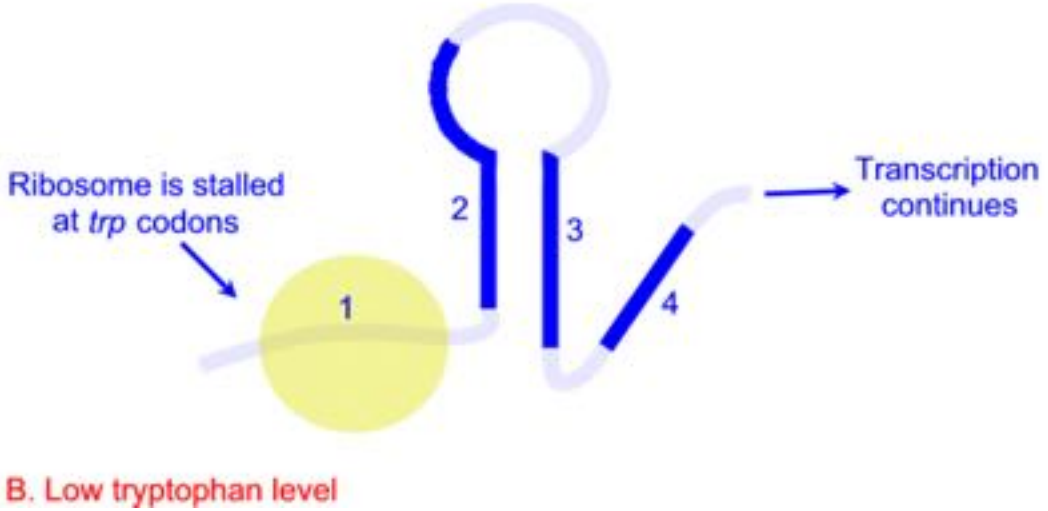
Tryptophan Biosynthese: Attenuation



Tryptophan Biosynthese:

Attenuation

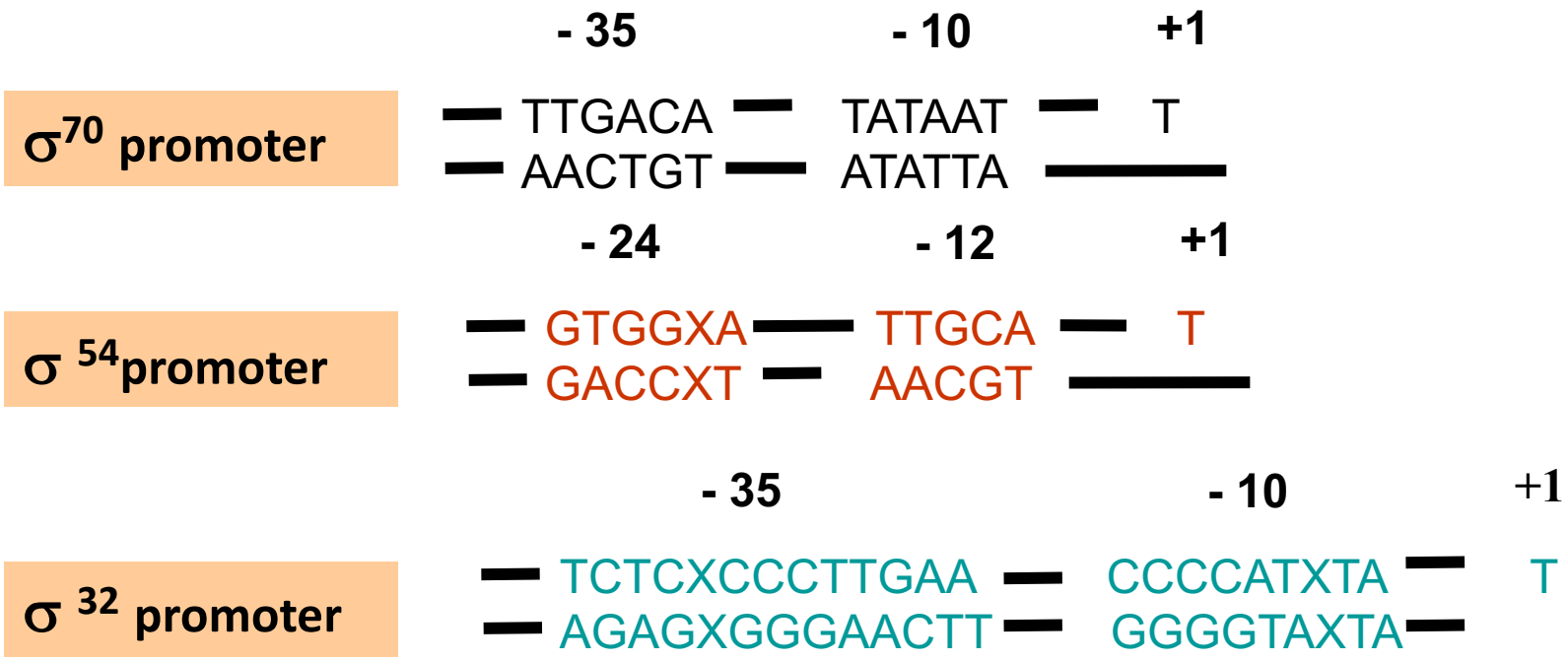




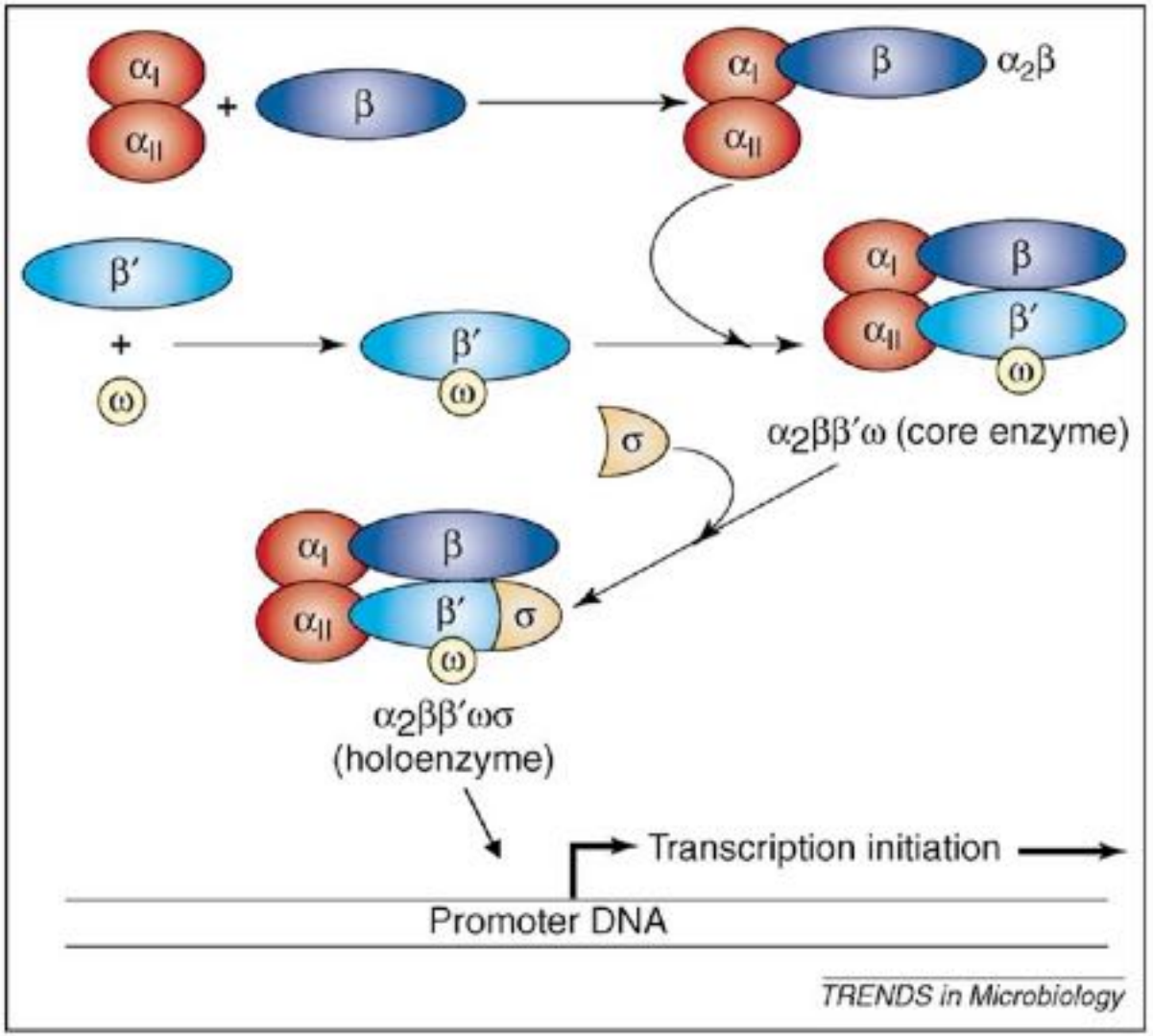
Promoter

1. CTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAAG
2. CTATTCCTGTGGATAACCATGTGTATTAGAGTTAGAAAACA
3. TGGTTCCAAAATCGCCTTTTTGCTGTATATACTCACAGCATA
4. TTTTTGAGTTGTGTATAACCCCTCATTCTGATCCCAGCTT
5. TAGTTGCATGAACTCGCATGTCTCCATAGAATGCGCGCTACT
6. TTGTTGACACCTTTTCGGCATCGCCCTAAAATTCGGCGTC
−35 sequence
Pribnow box
Consensus
TTGACA
TATAAT
Promoter sequence

Promoter-Sequenzen: σ 70, σ 54, σ32



Transkription - RNA-Polymerasen



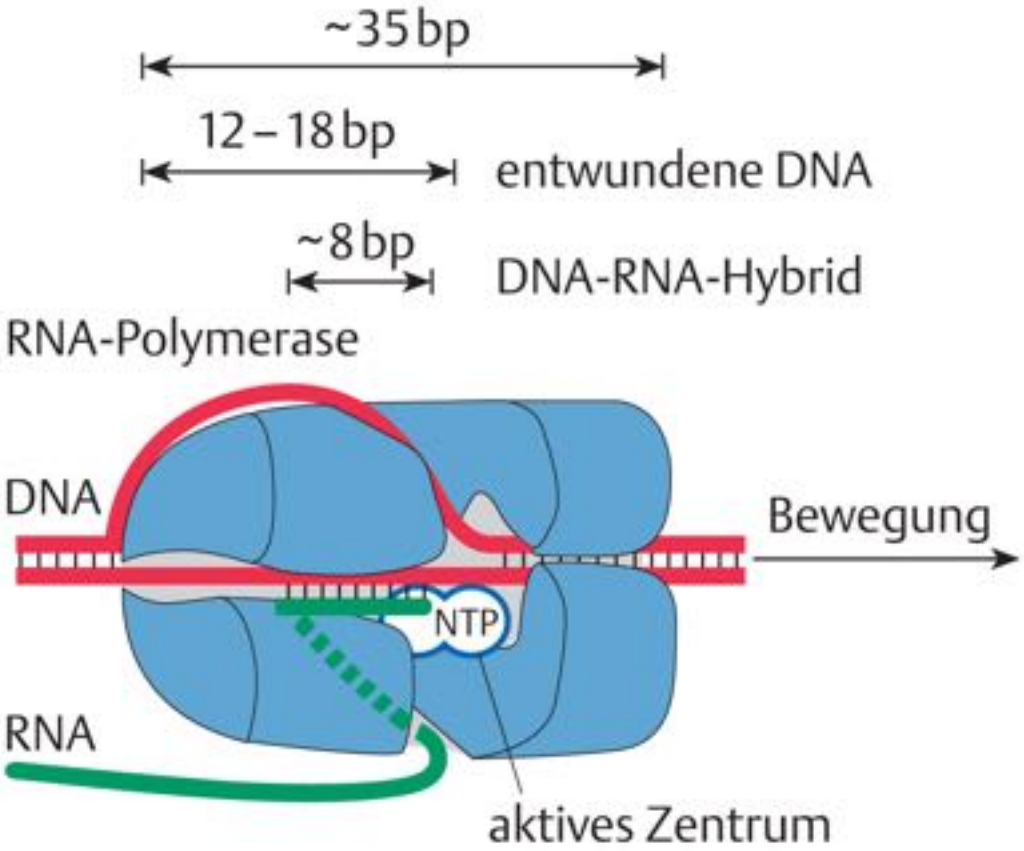
δ – Faktoren in *E. coli*

1. 70 Gene für exp. Wachstum (houSe keeping) (rpoD)
2. S (rpoS) generelle Stressantwort, stationäre Wachstumsphase
3. E Membranintegrität, Aufbau und Faltung von Membranproteinen
4. H (32) Heat shock, refolding von damaged proteins, Proteasen zum
5. Abbau von beschädigten Proteinen
6. FecI Gene für Fe Transport, Siderophore-Synthese, Fe-starvation
7. F (28) Gene für Flagellen-Synthese (fli, fla)
8. N (54) Stickstoffregulation

- Zahlen in Klammern bezeichnen die molekulare Masse

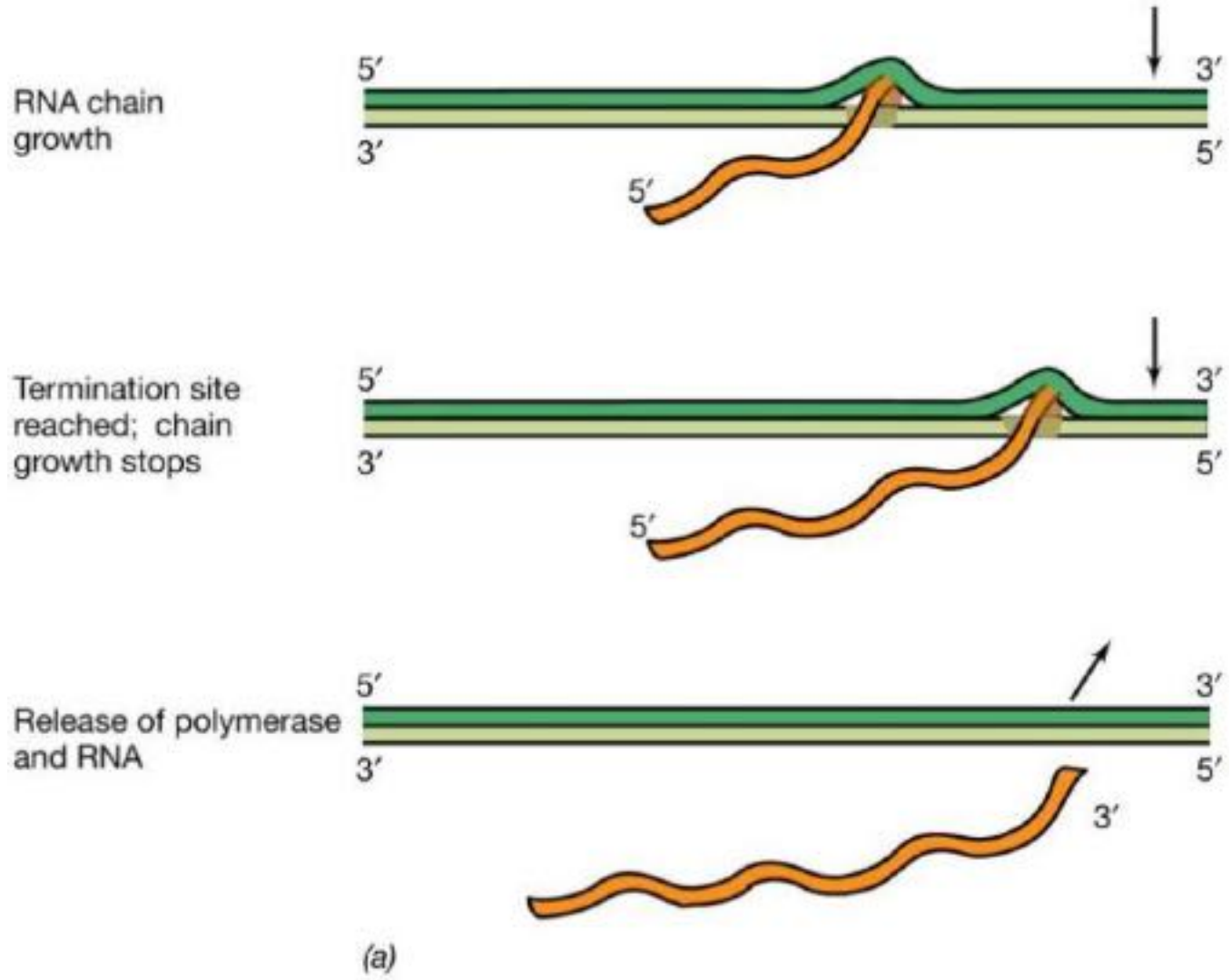
Transkription*sbubble*

Elongationsphase



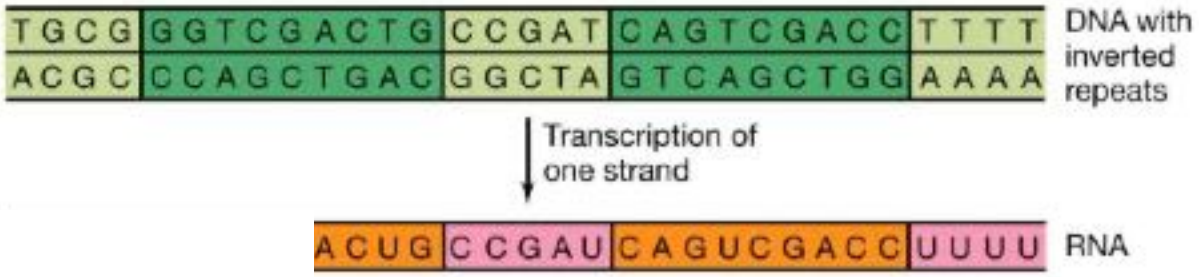
Elongation

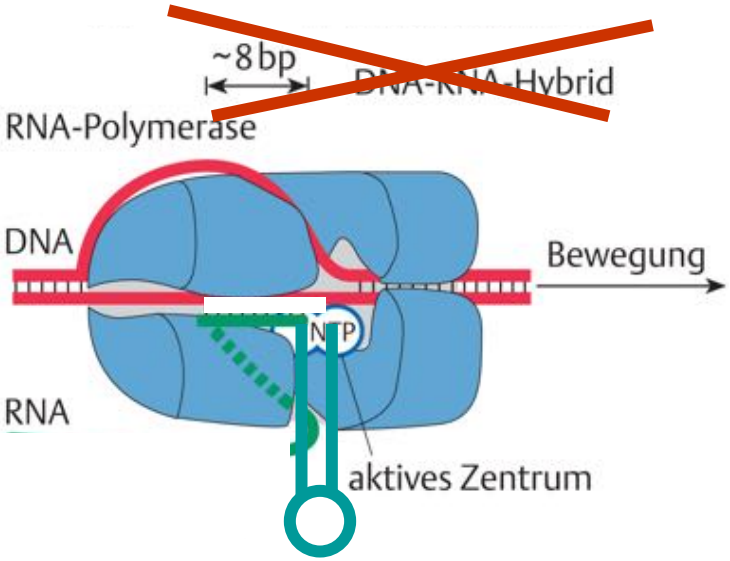
Termination



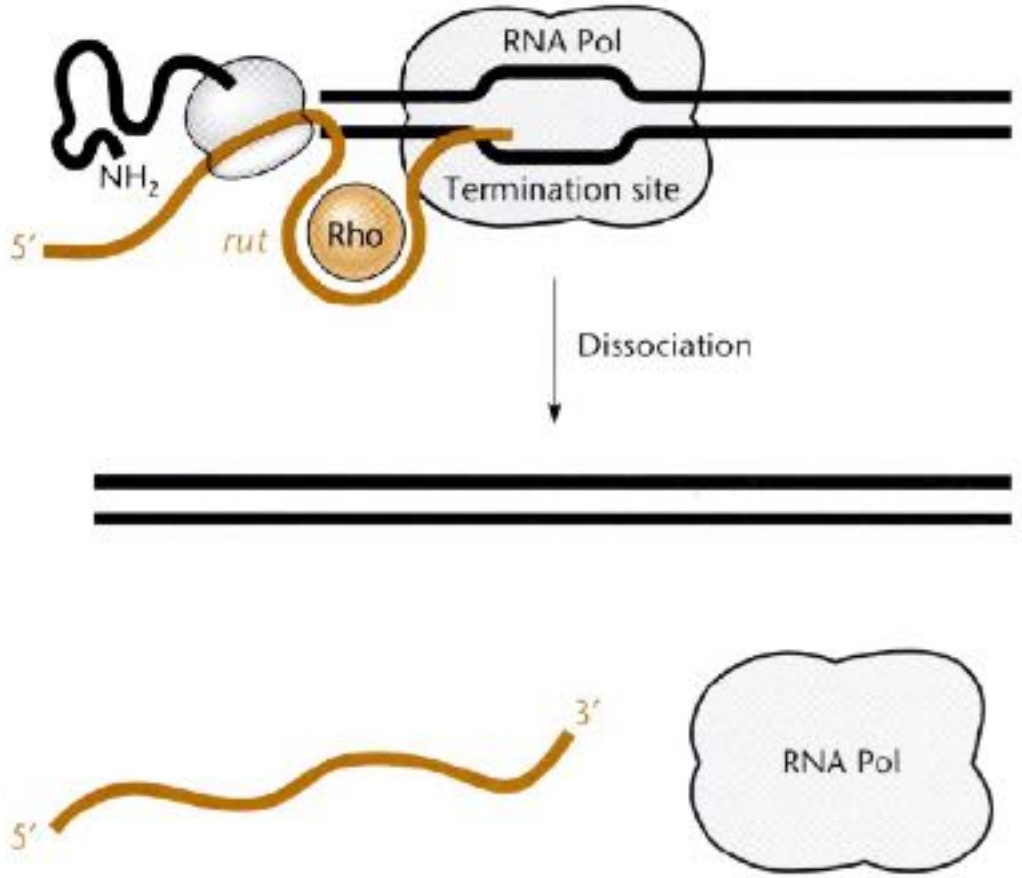
(a)

Faktor-unabhängige Termination

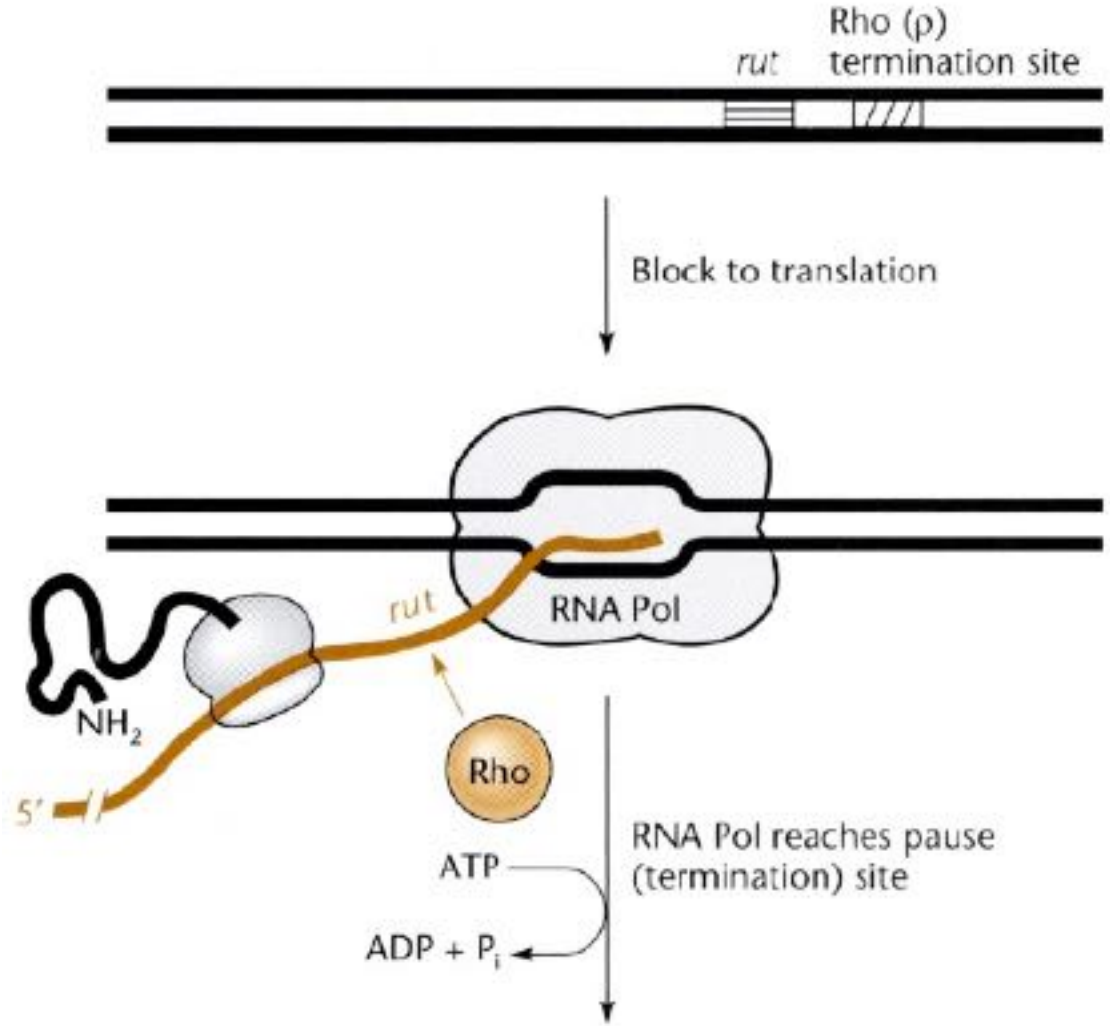




Faktor-abhängige Termination



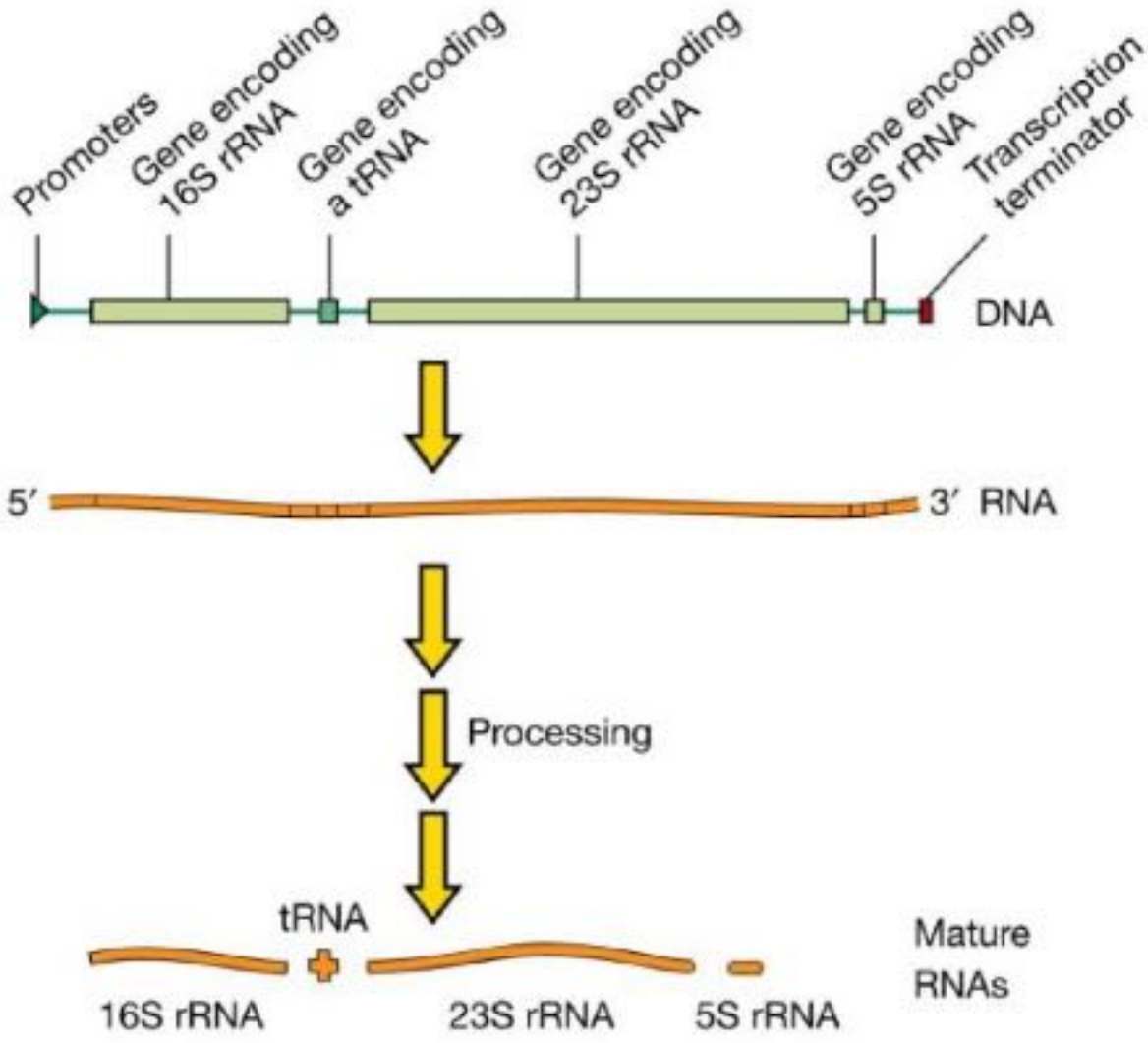
Faktor-abhängige Termination



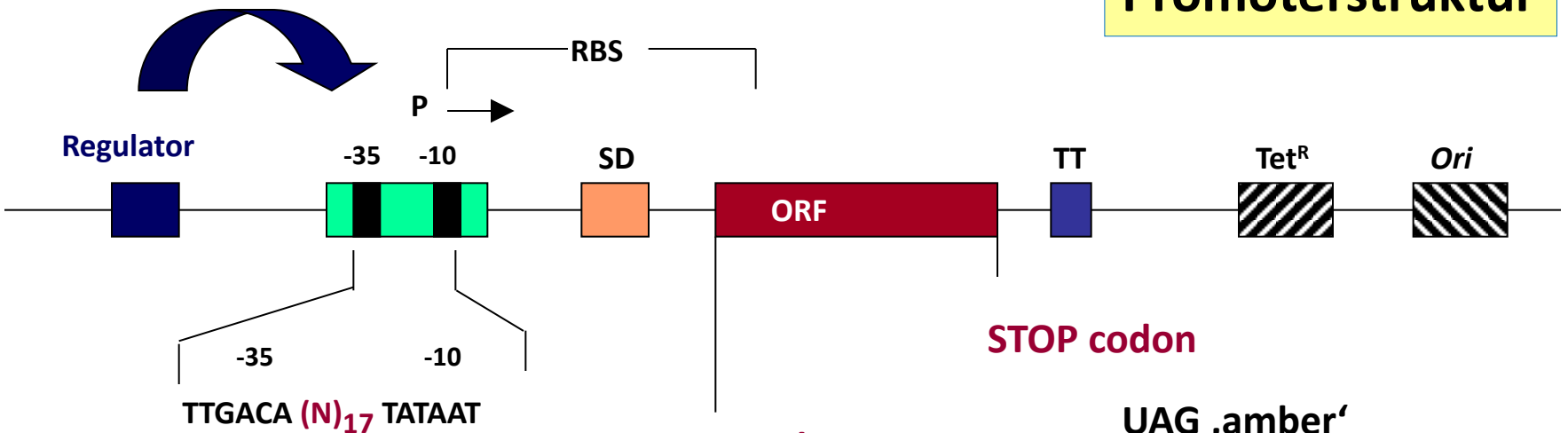
Nicht-translatierte RNA

rRNA, tRNA

Small RNAs



Promoterstruktur



STOP codon

UAG ‚amber'
UAA ‚ochre'
UGA ‚opal'

START codon

AUG (91%)
UUG (8%)
GUG (1%)